RAS MONOGRAPH SERIES
CARAMEL

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THE PHYSICAL AND CHEMICAL NATURE OF A CARAMEL COLOR
The Coca-Cola Company
Unpublished, 1953

A typical ammonia-sulfur dioxide caramel was found to contain 67.2 percent solids and 32.8 percent water. The ash content is of the order of 0.7 to 0.8 percent, the sulfur and nitrogen analysis on the well-polymerized fractions gives 1.05 and 1.72, respectively, or a sulfur to nitrogen percent ratio of 0.61. The reducing properties of the whole caramel are approximately 66.5 percent dextrose equivalents, while the highly polymerized portion is 37 percent dextrose equivalent on a dry weight basis. Alkali fusion indicates no sulfide-sulfur or primary amine.

The physical properties of caramel have been investigated by determining the molecular weights of important fractions, by measuring the properties of caramel in aqueous dispersion, and by determining the characteristic chemical group present which can produce ionization at several points in the full pH range. A number of different instrumental approaches to the properties of caramel have been made.

The molecular weights have been determined by measuring directly the diffusion coefficients of certain specific fractions of caramel fractionated by precipitation with

ethyl alcohol. From these diffusion constants and the Einstein equation, molar volumes can be calculated with the use of the measured partial specific volume. The molecular weights of the several fractions have been calculated both on the assumption that zero hydration and that 40 percent hydration takes place in the caramel molecule in aqueous solutions. According to these methods, the average molecular weights for 28 percent of caramel is 78,000, with 40 percent hydration; the average molecular weight is about 10,000 for 18 percent of the sample; about 5,000 for 23 percent; and the remainder of the caramel has a molecular weight of the order of 500. The molecular weight for 0.7 percent of caramel has been found by a very high speed centrifuge technique as being greater than about 1-million. Dialysis gives 75 percent dialysate, and therefore, 25 percent of the caramel has a molecular weight which is larger than 12,000. Freezing-point lowering on dialyzed materials gives very low molecular weights, in general, but greater than 2,000 have been found. All of these measurements indicate that caramel is a relatively highly-polymerized molecule, and some portions contain thousands of simple sugar units.

The visible spectra have been obtained, and at 500mμ, the coloring powers for all the polymerized materials are equivalent on a per gram basis. The ultraviolet and infrared

spectra have been determined and indicate large amounts of OH, no carbonyl, titratable OH groups probably of a conjugated structure, and possibly the presence of ether groups, all of which are consistent with our knowledge of the chemical structure.

The intrinsic viscosities have been measured on fractionated and dialyzed caramels and indicate that caramel is primarily spherical in nature and has the property of a dispersed polyelectrolyte, whose solubility in water depends upon the hydration of ionized polar groups.

The acid-base titration curve and the dilutions curve for caramel have been determined and indicate the presence of four definite reactions for which tentative structural interpretations have been made. The pK's of these reactions are 3.6, 6.1, 9.0, and 11.2, corresponding respectively to the sulfonic acid ionization, the quaternary ammonium ionization, the secondary amine dissociation, and, finally, the OH dissociation-ionization, respectively. The quantities of these materials present indicate that 70 percent of the sulfur is in the form of a titratable group, presumably the sulfonate group, while only 12 percent of the nitrogen present is in the form of a secondary amine. Apparently of the order

of one OH group is present per 6 carbons, and a formula
weight of 130 is found with $C_6H_5O_2(OH)$ $(S_{.043}O_{.073})$ $(N_{.16}H_{.16})$,
where the sulfonate replaces an OH, and the secondary amine
replaces an ether bridge. The dilution curve gives pK_1 slightly
greater than 3.6 and near 4.0. The two methods, however,
essentially substantiate each other. From these results,
the isoelectric point for caramel number 1 in the low pH
region is 4.9, at which point there are equal amounts of the
negative sulfonate ion and the positive quaternary nitrogen
ion. In the region of pH = 2.0, the positive quaternary
ion is present, while nearly all of the sulfonate ion has
been transformed to the ionized acid form.

A REVIEW ON THE FORMATION OF CARAMEL COLORS
The Sethness Products Co.
Unpublished, 1956

Caramel colors made from greens or refined sugars will have the following coloring matters (26):

A. Caramel substances resulting from heating the sugars with no nitrogen content.

B. Melanoidins due to condensation of reducing sugars with amino acids as N-glucosides.

C. Iron complex coloring compounds from reaction of iron salts with phenolic - OH groups of the humic substances causing an increase in color.

A. Caramel substances:

1. History

Caramel prepared from pure sucrose is a complex mixture made of sugar anhydrides (polymers). Its composition depends on time and temperature of heating (15) as seen below:

$$\text{Sucrose} \xrightarrow{\text{Heat}} \text{Non-volatile} \begin{cases} 1. & \text{Isocaccharosan} \\ 2. & \text{Caramelan} \\ 3. & \text{Caramelene} \\ 4. & \text{Caramelin} \end{cases}$$

Besides these non-volatile compounds, the following volatile compounds have been reported:
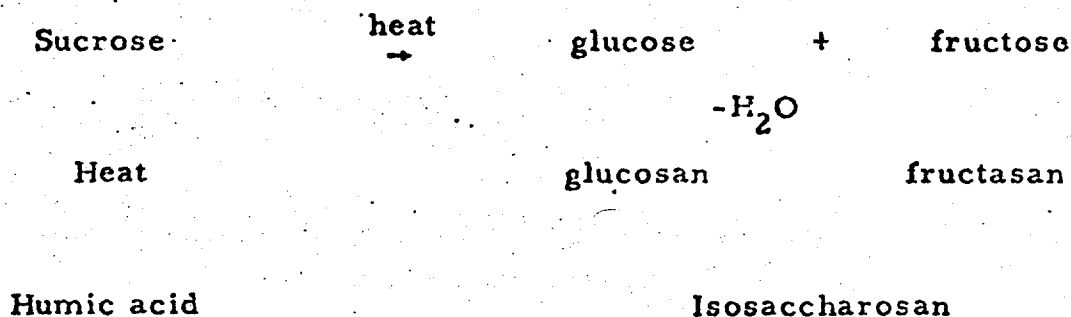
1. formaldehyde (2)
2. FORMIC ACID (22)
3. Diacetyl (19, 23)
4. furfural and hydroxymethylfurfural (23)

Note: 1) Humic precipitates are formed in non-pure sugars (1).
2) Methylglyoxal has been found sometimes and it is believed to be an intermediary in caramel formation.
3) Formic acid concentration is increased with increase in temperature of heating.

Treatment of caramelan and caramelene with HCl (conc. non-oxidizing acid) will give caramelin (2) which is a dark amorphous substance.

This disappearance of caramelan and caramelene and formation of caramelin has lead some workers to believe that caramelan formation is the first step in the process of anhydride formation and condensation which leads to humus and caramelin (2). Some believe that caramelin is an impure humin and that no caramelan or caramelene exist, while others think that caramelan, caramelene and caramelin are mixtures of humins and isosaccharosans (20). This last idea was supported by the following findings (15, 16):

Sucrose $\xrightarrow{\text{heat}}$ glucose   +   fructose

$-H_2O$

Heat   glucosan   fructasan

Humic acid   Isosaccharosan

Caramel color

Isosaccharosan has been prepared in the laboratory by reacting pure levulosan with glucosan (15). Humic acids are dark brown, alkali soluble, amorphous compounds that contain polymers of hydroxymethyl-furfural which could be split into formic and levulinic acids (15). The presence of isosacchorosans prevents the flocculation of humin (21). The ability of isosaccharosan to split into glucosan and fructosan has led some workers to believe that the colloidal humic substance is dispersed in two colorless substances that are of glucose and fructose derivatives (24).

The colloidal color, humin or caramelin, is a non-diffusable substance, that does not pass through a dialyzer, and its formula is a controversy between the different investigators.

2. Effect of type of carbohydrate used

Caramelization products depend on type of carbohydrate used. Caramelization of dextrose or invert sugar gives glucic and apoglucic acids in presence of alkali (16). Levulose caramel may contain levulinic acid, hydroxymethylfurfural and glucose (18).

3. Caramelization process:

The caramelization process is due to dehydration of the carbohydrates giving double bonds or oxide linkages and the products may be enols, ketones, furans, acetals and esters depending on the pH. In acid solution furan derivatives predominate, while in alkaline solution the process is mainly dealdolization (11).

The reducing power of sugars decrease during caramelization (1).

Caramelization of unrefined sugars is faster than pure sugars due to the presence of mineral compounds which cause higher degrees of dehydration (1).

The caramelization process is catalyzed by mineral acids, as H_2SO_4 and H_3PO_4 (8) and mineral salts especially the ammonium salts such as $(NH_4)_2CO_3$, NH_4Cl, $(NH_4)_2SO_4$ which give high amounts of color due to effect of ammonia (10). Na_2SO_4 and $Fe_2(SO_4)_3$ give higher amounts of color due to dehydrating effect (giving larger polymer molecules) and oxidizing effect of H_2SO_4, but the presence of Na_2CO_3, NaCl, KCl and Na-acetate give little coloration (17). In general these catalysts lower the caramelization temperature (9).

NH$_3$ is added to give a caramel color and the higher the percentage of ammonia added the higher tinctorial power (5). The mechanism is not well known. With ammonia, NaHSO$_3$ and Na$_2$SO$_3$ are added to buffer the mixture and maintain the desired pH in the caramel product besides the reducing power of NaHSO$_3$ (5, 13). It is believed that the preconditioning of the sugars with acids and ammonia prior to caramelization will bring the sugars into a form which permits caramelization to take place more along a chemical reaction than by "burning." of the sugar (13).

B. Melanoidins:

These N-compounds are the chief color contributors to beer and they increase the foaming ability of this product (4). Their formation can be blocked or hindered to a certain degree by SO$_2$ as in dehydrated food products (7). SO$_2$ blocks the carbonyl groups from reacing with the amino acids.

They can be formed from reaction of reducing sugars and amino acids or ammonia. Twenty-four or more definite compounds have been found in glucose-glycine reaction and about eighteen compounds in a glucose-aqueous ammonia solution (7). Ammonia reacts easily with glucose and much faster with fructose giving mixtures of ketoses and aldoses (12).

There is a great similarity between melanoidin and humic acids (3). Both are acidic in nature and possess a great buffering capacity (4). They act as a protective colloids.

Caramel colors prepared by heating concentrated sugar solutions exhibit behaviors similar to proteins in solutions, as colloidal material, and possess a certain isoelectric point (25). The isoelectric point of caramel is fixed during its manufacture and cannot be changed simply by adjusting the pH after the process is finished.

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CARAMEL COLOR

#4681

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PART I

CARAMEL, FLAVOR and COLOR

Caramel is defined as the concentrated aqueous solution of the product obtained by heating sugar or glucose until the sweet taste is destroyed and a uniform dark brown mass results, a small amount of alkali or alkali carbonate being added while heating, (n.F). Possibly a borader descriptive definition is that caramel is the mixture of products produced by heating sucrose,glucose,molasses or other saccharine products within the range of 190-220 deg. C. It should be noted that the Food and Drug Administration deems caramel to be an artificial color since it is made by the artifice of partially breaking down sugar.

Properties:

Caramel is a dark brown to black,viscuous liquid with a characteristic odor of burnt sugar from which property it gets its synonyms of Burnt sugar and burnt sugar coloring. It has a pleasant, somewhat bitter taste, Caramel(N.F.VII) should have a specific gravity not less than 1.30 at 25 deg. C. It is soluble in water and one part dissovled in 1000 parts of water should have a clear sepia tint. Such solutions should not change perceptibly within six hours nor should any precipitate from within this period on exposure to sunlight. Caramel is soluble in water in all proportions and is also soluble in alcohol solutions containing less than 55 percent by volume but it is insoluble in most of the common organic solvents such as ethyl ether, petroleum, benzene, chloroform, acetone and the like.

As detailed below, caramel is composed of a mixture of substances. When prepared from pure sucrose caramel is completely soluble in water. However, when prepared from unrefined sugar certain water-insoluble components are formed. The quantity of these insoluble components increases with increase in the extent of dehydration. The caramel made from sucrose is much more soluble in aqueous alcohol solutions than that made from commercial glucose for the latter contains dextrins which on heating form alcohol insoluble derivatives. The addition of alkali carbonate during the heating process apparently increases the coloring power. Caramel acts as a stabilizing colloid in molasses and syrups but apparently does not affect the viscosity.

Solid and powdered caramels are also avilable. Solid caramelis generally a reddish-brown, brittle, amorphous, highly deliquescent material.

Composition:

While the chemical composition of caramel has not been entirely elucidated, investigators over a period of years have been able to gather certain information concerning its composition. When sugar and the other saccharine products mentioned above are heated above their melting points but below the point at which they are likely to char, a sticky deep brown-colored maws is obtained. This is a mixture of various components which are termed various by different investigations of dehydration products, decomposition products, etc. Undoubtedly some of the components are simple dehydration products of the sugars; others are polymerized mono-and disaccharides. When the starting material for the preparation of carmel is a chemical compound such as sucrose or D-glucose, the mixture produced by the heating process is less complex than when the starting material is a mixture such as molasses, commercial glucose and analogous materials.

One of the first investigators was Gelis (1851-1862) who concluded that caramel from sucrose consisted of three products, one a dehydration product caramelan $C_{12}H_{18}O_9$, equivalent to a 12 percent loss in weight, and two polymers Caramelan $C_{36}H_{50}O_{25}$ equivalent to a 14-15 percent loss in weight; and Caramelin $C_{96}H_{102}O_{51}$, equivalent to a 20 percent loss in weight.

Ehrlich deemed the coloring matter of caramel from sucrose to be a compound $C_{12}H_{22}C_{11}$, $\neq 2H_2O$ which he named saccharine. He obtained this material by heating sucrose under vacuum in a flask immersed in oil at about 200 deg. C. The residue remaining after extraction of other materials by methyl alcohol was dissolved in water, filtered, and evaporated to yield a dark-brown amorphous material readily powdered. Browne and Zerban call this material Saccharan. One part of this material colors 10,000 parts of water a deep brown which can be intensified by the addition of alkalies. It is tasteless and is not precipitated by lead subacetate in contradistinction to other coloring matters in syrups. Meade, however, found that impurities in molasses and analogous products when precipitated by lead subacetate and even lead acetate, coprecipitate the caramel. Later workers in this field confirmed the fact that caramel is a mixture. Cunningham and Doree isolate caramelan as a buff colored powder, melting at 136 deg. C. and found by molecular weight

determinations that it is probably a dimer with a formula $C_{24}H_{36}O_{18}$.

By heating sucrose under reduced pressure for 15 minutes at 135-190 deg. C., Pictet and Adrianoff obtained in addition to caramelan and caramelin described above, a compound which they termed isosacchararosn $C_{12}H_{20}O_{10}$. This material, precipitate from methyl alcohol by acetone, is a bitter tasting, very hygroscopic, water-soluble powder melting at 94-94.5 deg. C.

Separation of Components:

Gelis noted that caramelan, the simplest of the dehydration products, was soluble in 64 percent alcohol; caramelan was soluble in cold water but insoluble in alcohol; and caramelin was insoluble in cold water. Garino and Tosonotti made use of these differences in solubility in separating these components. Dialysis methods were used by Janacek to separate these components. His work indicated that the Formula $C_{125}H_{188}O_{80}$, assigned to caramelin by Sabaneey and Antuschevitsch was probably a better representation than that assigned to this compound of Gelis.

Utilization:

Caramel has wide utilization in the flavor, non-alcoholic and alcoholic beverage, baking, frozen dessert, and confectionery industries both as a color and as a flavoring agent. Its use, for instance, in imitation vanilla flavor is in an outstanding example. It is used in medicine principally as a coloring agent.

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PART II

PREPARATION OF CARAMEL
Morris B. Jacobs

Manufacture:

Sucrose:

A caramel can be made from sucrose merely by
heating it above its melting point, 186 deg. C., but below
the point at which it chars.   Such a method is inadequate for
commercial production.   A relatively simple formula is to heat
100 pounds of simple syrup to boiling, add about 150 grams
of 28 perc nt ammonia water while mixing the batch, and add
an aqueous solution of ammonium carbonate containing 250
grams of the salt.   The heating is continued until a tempera-
ture in the range of 350-450 deg. F. (176.6 - 232 deg. C.) is
obtained.

An alternative method is to heat a solution of
sugar in concentrated ammonium hydroxide solution under pres-
sure at 105-120 deg. C.   Any volatile products formed in
the process may be removed subsequently by passing steam
through the product.   Pressure may be released and reapplied
in the processing step.

Specific recommendations for the preparation of
caramel have been given by Leopold and by Sollich.   The
former investigator found that it is best to heat the sugar
at 177 deg. C. for 16.5 minutes and that longer heating causes
the development of a bitter taste.   The letter investigator
found that partial concentration was best performed in Vacuc.
Still another recommendation is to heat sucrose at 210 deg. C.
It is known that unrefined sucrose carmelizes much more readi-
ly than refined sucrose.   This is attributed to the mineral
content of the unrefined product.

Raw Sugar or Molasses:

One method of preparation using these sources of mater-
ials follows the procedure outlined below: Dilute the raw
sugar solution or the molasses and allow to stand until virtu-
ally all the suspended material has settled out.   Invert the
sucrose with sulfurous acid and remove the excess sulfur diox-
ide by boiling for 0.5 hours.

Neutralize with lime and filter.   Boil the filtrate cautiously
using a vessel equipped with a steam coil.   Replace the water
lost during boiling and continue the process until a pro-
duct of adequate coloring power is obtained.   Evaporate to a
viscous consistency.

Starch, Dextrin and Glucose:

Caramel may be prepared from starch by several
methods.   In Europe a common procedure is to dampen powder-
ed starch with dilute sulfuric acid. The batch is heated
to 100-120 deg. C. and is held at that temperature until
hydrolysis of the starch and dextrin to glucose is complete.
This may be checked by the addition of 95 percent alcohol
to a solution of a sample of the batch.   A clear solution
indicates complete hydrolysis: a turbidity indicates the pre-
sence of dextrins.   Calcium carbonate is added to neutralize
the sulfuric acid.   The mixture is added to neutralize the
sulfuric acid.   The mixture is allowed to stand; the clear
supernatant liquid is decanted from the precipitated calcium
sulfate: it is concentrated to 36 deg. Be.; and is filtered.
After transfer to a vessel the hot filtrate is heated to boil-
ing while being stirred.   Sodium carbonate is added in the
ratio of 3 parts to 100 parts by weight of the batch and
after the color deepens, the heat is reduced gradually to
avoid carbonization.   Partial cooling is accomplished by addi-
tion of hot water.   The caramel is extracted with water, fil-
tered, and then diluted to provide several grades of color.

In an alternative procedure of preparing caramel from
starch, the starch is saccharified by heating with dilute
hydrochloric acid under pressure.   The sugar obtained is sub-
sequently heated at 120-130 deg. C. with a catalyst such as
ammonium chloride, aluminum sulfate or calcium chloride.

In one process the caramel is made directly from
dextrin without conversion to glucose.   The starch is con-
verted in customary ways to dextrin and the latter is carmelized
directly in the presence of ammonia or other alkali.

Di Baja made a caramel paste from glucose by
hydrolized potato and corn starch in an autoclave with dilute
acid.   He stressed the importance of quality in the glucose
used, recommending 41 percent as the minimum, D-glucose con-
tent, required in such commercial glucose.

Malts are specially prepared to provide caramel coloring. Ordinary malt is given an additional processing by drying, steeping, and heating until it is adequately caremlized. The color of these products ranges from yellowish brown to a blackish brown, the latter being produced by high temperature drying. In this instance, the principal sugar being carmelized is maltose.

Lactose:

While this sugar has not been used to any great extent for commercial caramel production, it is to be noted that it loses water of hydration at about 130 deg. C., and while melting at 200 deg. C. undergoes caramelization at 160-180 deg. C.

Soybean Carbonhydrate:

The crude starchyose obtained from the by-products in the extraction of soybean oil by alcohol can be hydrolyzed with dilute hydrochloric acid; the acid concentration can be reduced to hP 2.3 and then the material can be hydrated by heating at 130 deg. C. While crude starchyose could be used, a commercial caramel could not be prepared by this investigator by the direct heating of starchyose.

Instead of using crude stchyose the lower layer of soybean oil foots, after treatment with acid can be used for it is rich is sugars. This layer is filtered and heated at 130 deg. C. The caramel solution is neutralized with sodium hydroxide to a pH 6.0 - 7.0. The bitter taste of the caramel preparation was removed by the addition of a small amount of alcohol or by the addition of disodium hydrogen phosphate, monosodium dihydrogen phosphate, or trisodium phosphate.

Caramel Powder:

To prepare a caramel powder the following procedure may be employed. Decrease the viscosity of the caramel by adding suffucient water to make the total solids content of the mixture 60 per cent. Reduction of viscosity can also be achieved by heating the caramel to approximately 85 deg. C. -The fluid can then be spray evaporated at 40-50 deg. C. with preheated air.

A caramel powder mixture for use in foods can be made by taking dry, finely powdered caramel and mixing it with sufficient sugar so that it will not cake when the mixture is kept in a dry, air-tight container under commercial storage conditions.

The time and temperature of heating sugar masses containing water and an alkaline substance, can be arranged so that caramelization and evaporated to dryness occur simultaneously.

Utilization:

Caramel is used widely as both a flavor and coloring agent. A principal use is in imitation vanilla flavor where it is commonly employed in the ratios of 7 to 10 parts of caramel per 1000 parts of total ingredients. At other times caramel is added to suit the taste of the processor. This practice is undoubtedly attributable to the desire to obtain a product looking like natural vanilla flavor but undoubtedly the caramel adds a note of its own.

Caramel made from starch by the sulfuric acid method detailed above is commonly used in Europe for coloring alcoholic beverages such as beer, brandy, and high wines. An equally common practice is to use caramel color in rum. Indeed virtually all rum from Jamaica is colored with caremel. For coloring beer and wine it is not necessary to free the glucose, used as the raw material completely from dextrin. In addition ammonium carbonate may be used as the alkali instead of sodium carbonate.

Caramel malts are used for coloring whiskey, beer and sometimes vodka. Caramel, however, is employed not only in alcoholic beverages but also in nonalcoholic beverages like soda pop.

In addition to its use for the products mentioned above, caramel is often used for the coloring puddings, cakes, syrups, ice cream and certain medicines.

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PART III

PRODUCTION OF STABLE CARAMELS FOR THE FOOD

AND BEVERAGE INDUSTRIES

Where invert sugar is also manufactured, it may be convenient to use invert for caramelization, as the invert itself can be produced in the form which experience has shown to be the most suitable. To make a good caramel, the best and purest No. 1 invert must be used.

In the food industries (e.g. for soups, pickles, gravy browning, cakes, etc.) it was once thought that the only requirements of a caramel were that it should have the maximum possible color compatible with reasonable fluidity; however, the complete picture is not quite so simple as that. Although none of the products for these purposes has to be bright in solution, if an unstable caramel is used, it is found that the % color recovery at the final stages is deficient.

In very general terms, "bad" caramel can be defined as that in which, during manufacture, the colloidal particles have been allowed to grow in such an uncontrolled manner that they have ceased to be colloidal at all, and, as soon as the caramel is diluted, even with pure distilled water, precipitation takes place.

An unsuitable caramel is one of which the isoelectric point is such that, at the pH of the product in which it is to be used, it carries an opposite electric charge from that of the colloids already present; when this happens, the colloidal particles attract each other into increasingly unstable aggregates, with eventual precipitation. A product the particles of which are negatively charged must always be colored by a caramel with the same characteristic. It will be appreciated, therefore, that the inability of a good caramel to color a product for which it is unsuitable is no reflection on its inherent merit for its proper purpose; nor is it any good discussing iso-electric points if the caramel is inherently unsound.

Precautions must be taken, therefore, in the first instance, against "forcing" and "stewing." The caramel pans are accordingly equipped with recorder charts, from which the whole time-temperature performance of the batch can be observed. Experience has shown that the almost invariable cause of a batch requiring pushing along (being reluctant to take color) is inefficient absorption of the alkaline catalyst. This is reflected in the final nitrogen figure, which is therefore checked on every batch and written on the recorder chart for reference. Additionally, the pH of the liquor is checked in the laboratory before acceptance for transfer to the caramel pan. Beer caramels are, almost universally, straight liquid ammonia caramels nowadays, with transfer to the finishing pan at a pH value of 8.5 and aiming at a final nitrogen (for 50,000) of 4.2%. Incidentally, it seems illogical to express this in the form "N X 6.25" as protein.

At the present time there are two methods of getting the ammonia into the invert sugar, a process which depends on a very precise control of time, temperature and contact. It is not quite clear which method is the better. In one, the ammonia is gassed into the sugar in a tall slender tower called the bubbler, the rise in temperature in this device being controlled by a large cooling coil. On the whole, there is fairly good absorption. In the other system, the ammonia is stirred very gently into the sugar with a slow-speed stirrer in a large and comparatively shallow pan fitted with both heating and cooling coils, and by a touch of the control valves, the operator has absolutely instant temperature control.

The third danger point with caramels is "working on". To obviate this, there are a variety of methods of progressively "killing the heat" as soon as the final peak temperature is reached. The first is the application of quench water, which is sprayed over the caramel from numerous fine nozzles, rather like a sparge arm. This converts the product, which would otherwise soon solidify, to normal pumpable consistency, and at the same time causes a fall in temperature of about 30 deg. C. While quench water is going on, the operator turns on the cooling water, which with the older type of gas-firing pan goes into an elaborate trombone-shaped coil and with the more recent type just floods the jacket. Meanwhile, the rouser continues to revolve, and after about 2 hours the temperature is down to 65 deg. C. The batch in then dropped and pumped to storage vats, in which a small cooling coil operates, and at about 40 deg. C. the caramel is safe to rack.

Even now the danger is not entirely passed, for if it is subsequently heated for any length of time the caramel will start to work again and its beer stability is lost. The first test applied to any allegedly defective caramel is to check its color power to see if it has risen above normal. Needless to say, this problem is a very real one in the export trade where goods have to pass through or are used in the tropics. Means are constantly being sought for the improvement of caramels from a "reheating sensitivity" point of view. Actual trade packages are incubated at 60 deg. C. and the contents are examined once a week for three months.

As soon as the quench water is thoroughly mixed in, a sample of the caramel is drawn for the laboratory, where the color is checked and nitrogen and beer tests are put on. Colors are actually read on a photo-electric colorimeter. The beer test consists of taking a measured quantity of de-gassed and filtered beer, coloring it to a standard tint and leaving for 24 hours. It is then examined against the uncolored control in what amounts to a 4 in. cell in scattered light. The instrument used is a type of "Tyndallometer" and is very effective and severe in action.

The iso-electric point of beer colloids is at 5 pH and that of a typical beer caramel is at 6.9; the normal pH of both being in practice below their iso-electric points, they are positively charged. By contrast, the iso-electric point of certain types of soft drinks is below 2.5, and of special caramel for such drinks below 1.5 ; both, in the finished product being at a higher pH, are therefore negatively charged. The wrong caramel, either way round, would be equally useless. It should be emphasized that the iso-electric point of a caramel is fixed during its manufacture by the nature of the alkaline catalysts used and by the time-temperature cycle followed; these factors also govern the final pH.

Altering the pH of the caramel after manufacture does not alter the iso-electric point. Beer caramel may have a final pH of 4.6, and soft drinks caramel one of 3.2; raising the pH of the latter to 4.6 would not make it a beer caramel, nor would lowering the pH of a beer caramel to 3.2 make it any more suitable for soft drinks containing vegetable extractives. The only result of neutralizing a caramel, apart from making it less acrid, is to render it more liable to mould growth.

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PART IV

CARAMEL IN THE BREWING INDUSTRY

In the last two decades increased attention has been given by brewing biochemists to colloids of wort and beer. The extensive studies on wort proteins, such as the recent work by Sandegren and St. Johnston for example, is rapidly pointing the way to satisfactory understanding of the function of some of the most important nitrogenous colloids in wort and finished beer.

Melanoidins and Caramels in Brewing:

A distinctive group of substances which should be of great importance and interest to the brewing industry, namely, the melanoidin and caramel group has been little investigated. For lack of precise information concerning the structure of these substances they can be grouped together. Melanoidin materials are usually regarded as the brownish substances resulting from the chemical combination of carbohydrate materials with amino acids or ammonium salts. "Caramel" usually indicates substances produced when carbohydrate materials react with ammonium compounds, or those obtained when sugars and other carbohydrates are heated to temperatures high enough for considerable decomposition to occur. It is almost certain that the principal colored substances present in wort and beer do contain more or less nitrogen.

So far very little is known of the chemistry of the melanoidins and the caramels. The amount of natural coloring matter present in a wort depends on a number of factors, including the nature of the original grain, the time, temperature, and pH of the malting and mashing processes, composition of the steeping water, pH of kettle wort, boiling time, variety and quality of the hops used, oxidation-reduction conditions occurring in malting, mashing, boiling and subsequent cooling stages, and probably many other factors. Much color production takes place during wort cooling, probably because of oxidative changes. The final color of a pale beer will depend on all these points.

Much of the color of the natural boiled wort is often filtered out before fermentation; some color is deposited upon and precipitated along with the yeast. Very little has been

done to separate and characterize these natural melanoidins of wort, but they appear to display many of the properties of the caramel colorings which are discussed below. Some of the melanoidins present in wort and beer, and in other important raw materials such as various kinds of molasses, are subject to precipitation when the pH of the medium is lowered. In this way they differ from the coloring matter of good beer caramels which should exhibit considerable stability when acids are added.

Manufacture of Caramels:

The manufacture of caramel colorings from carbohydrate materials for various kinds has been a large and important industry for many years but very little has appeared in the technical literature about the methods of preparation or the properties of these materials. Caramel is used to color a wide variety of foods and beverages including bakery products, beers, wines, soft drinks, cider, vinegar, spirits, gravy colorings, and many more. Many hundreds of tons of these coloring materials are produced yearly in England, the United States, and other countries. They are prepared by heating very concentrated solutions of invert sugar, glucose syrup, or other sugary materials with ammonia gas or various ammonium salts, with or without other inorganic substances which act as "catalysts" in production of color. The heating often up to temperatures of 120-130 deg. C. is carried on until the color produced is satisfactory. The mixture is then rapidly cooled and run into suitable packages. By "rule of thumb" techniques built up over the years, caramel materials of good coloring properties have been produced, but it is fairly certain that few scientific principles are known concerning the methods of preparing them.

Properties of Caramels:

A careful check of the various types of caramel coloring materials available commercially in recent years has been made. This has shown that commercial caramels vary widely in some of their properties. For example, the coloring power of available caramels varies from 20,000 to 60,000 color units, as measured in degrees Lovibond (series 52) and estimating the color from a 0.05 per cent solution of the material in a 1-inch cell. The flavor also varies from a bland and sweet material to a harsh and bitter flavor almost entirely free of sweetness. The consistency of some caramels is such that they flow easily; others have hardly any "flow" and many easily

solidify in their containers.   Ash content varies from 0.3
per cent to more than 4 per cent, and the pH of a 5 per cent
solution of the caramels in distilled water may be anywhere
between 4.0 and 6.5.

Colloidal Properties:

Any of these properties may have an important
bearing on the choice of a caramel coloring for a particular
purpose, and users like to specify clearly defined values for
their own especial uses.   An extensive examination, however,
has revealed that by far the most significant property of a
caramel is its colloidal behavior in solution.   We have been
able to demonstrate that the color particles of a commercial
caramel in solution exhibit electrical behavior and that the
dissolved particles can carry positive or negative electric
charges, or some of them may be neutral in behavior.   The
exact charge depends on the pH value of the solution of the
caramel, and the value and nature of the electric charge can vary
with pH.   Thus the caramel particles behave in a manner almost
identical to that of dissolved protein molecules, and posses
clearly defined isoelectric points at which the electric
charge is minimal.   At pH values on the upper (alkaline) side
of the isoelectric point the particles are negatively charged;
on the lower (acid) side of the isoelectric point the charge
on the colored particle becomes positive.'   The value of the
electric charge becomes increasingly negative with rising pH
and increases positively with lowering of pH.   It appears
likely, therefore, that "salts" of the caramels are formed
with alkalies and acids, and that these might be described as
"sodium" caramelate and caramel "sulphate."   This is similar to
the formation of "sodium" albuminate and albumin "sulphate"
by albumins in alkalis and acids.

The similarity of caramel to protein in behavior is
very great indeed, and many of the protein properties investi-
gated and described by Loeb in his classical investigations are
shown by caramels.   Loeb demonstrated that the charge on protein
particles (as well as on their osmotic pressure and other pro-
perties) is minimal at the isoelectric point of the protein,
but that the charge increases with decreasing pH (increasing
acidity) to a maximum, usually at a pH of about 3.0.   At pH
values below this point the charge steadily falls because of
the depressing effect of the acid anions that accumulate in
the solution.   With one caramel coloring with an isoelectric
point at pH 7.0, which we have investigated, it was found that
the caramel particles became increasingly positively charged
down to a pH of about 2.85, but the charge became less posi-
tive at pH values below 2.85.

The effect of solutions of neutral salts on proteins was also investigated by Loeb, who showed that low concentrations of these salts, by yielding charged ions to the solution, depressed the charges on colloid particles which carried electric charges of opposite nature, in this way depressing the various colligative properties of the protein in solution. In similar fashion it has been found that neutral salts depress the value of the electric charges on caramel particles in solution. This is a most important reaction and may well tend to mask the true colloidal nature of the caramel.

Two Classes of Caramels:

Examination of many commercial caramel colorings has shown that, broadly speaking, they fall into two chief classes. The first of these is definitely characterized by possession of a large amount of colloidal coloring with an isoelectric point in the region of pH 7.0. The other group has weaker electrical properties, but is characterized by a color with an isoelectric point at about pH 3.0. This does not mean that all the coloring materials in these commercial caramels have these properties. There is, undoubtedly, a part of the color which is neutral in electrical reaction and this uncharged fraction is very desirable since it will not disturb the colloidal balance of any system to which it is added. The electrically charged coloring matter, however, can greatly influence a system to which it is added, as will be explained later.

In our experience the caramel with coloring of a high isoelectric point (pH 7 or thereabouts) is obtained commercially by the interaction of ammonia, and invert sugar or glucose, followed by heating to complete color production to the required degree. At ordinary temperatures, the resulting substance is a reasonably freely-flowing material with a coloring power between 20,000 and 60,000 units (normally 40,000 to 60,000). It contains up to 3.5 per cent of total nitrogen, none of which is freed as ammonia by heating with alkali, and usually contains but little ash (up to 0.5 per cent.) The preparation of such caramel has been described by Comrie. (1).

There is available in large amounts, however, a caramel
which has an isoelectric point at about pH 3.0. This is a
most important material for certain industries that find the
higher pH isoelectric point type quite unsuitable for their
particular needs. It is difficult to obtain this material
with a coloring power much higher than 27,000 to 28,000 units.
Our experiments have shown that it is produced by heating
invert sugar or glucose with small amounts of ammonia in the
presence of some suitable catalyst such as sodium hydroxide.
Of considerable interest is the fact that the material is
often of a thick consistency and that it contains a large amount
of ash (3 per cent or more), and normally only about 0.3-0.6
per cent of nitrogen, all of which is tightly held and cannot
be released as ammonia by alkaline treatment.

It cannot be too strongly emphasized at this point
that not all the coloring matter of these two groups of cara-
mels is always electrically charged. Usually it is a fraction
of each type which carried the charge and gives the material its
characteristic properties.

Caramels for Brewing:

If confronted with both types of caramel, which type
should the brewer use for coloring his beer? The product of
the high isoelectric point is high in coloring power but its
flavor is usually rather acrid, and it is not very sweet. On
the other hand, though the material of low isoelectric point
has a coloring power of only 22,000 to 28,000 it is much more
bland in flavor and is sweeter, since less of its sugar has
been "burned" into color material. Experience has shown, how-
ever, that the material having the higher isoelectric point
is by far the most suitable for brewing purposes. The use of
this material usually yields beers which are bright, clear,
and sparkling and which maintain this appearance for consider-
able periods on storage, except for production of chill haze
and possibly of certain of the normal hazes such as metallic
hazes.

Wort and Beer Colloids:

A caramel containing color having an isoelectric
point at about pH 3.0, however, is often quite unsuitable for
coloring of beer as its use is accompanied by the production
of heavy deposits and hazes. The behavior of the various types

of caramel in malt wort depends on the reaction which the electrically charged color colloids have on the wort colloids. Although much work remains to be done on the colloids of wort and beer, St. Johnston (2) has demonstrated the presence of four protein fractions which he has designated T, C, D, and O in sweet wort and boiled hopped wort. Of these, protein T is a globulin-tannin complex, having an isoelectric point at about pH 6.4. Protein C has an isoelectric point at about pH 6.0 and yields protein O upon denaturation and oxidation. Although protein C is coagulated by boiling, St. Johnston regards such coagulation as rarely complete, owing to oxidation to protein O, which appears as a dark residue in solutions exposed to air. It has an isoelectric point at pH 3.9, at which it is precipitated. St. Johnson states that numerous turbidities and flocculations in the brewing and keeping of beer can be accounted for by the mutual precipitation between the negatively charged protein O and the other positively charged proteins (3). He also regards protein O as similar to the wort nucleo-protein isolated by Hopkins, Amphlett, and Berridge. (4). Hopkins has also described a heat coagulable protein of malt wort, fully precipitated at pH 3.8-3.9, probably a nucleo-protein. This could be prepared without any color (5). If this protein and St. Johnston's protein O were identical, protein O would be impure, possibly by contamination with adsorbed color.

Sandegren has described one albumin and four globulin fractions obtained from barley protein. The albumin and one of the globulins he singled out as of particular interest in brewing. Lloyd Hind (6) discusses the presence of amphoteric protein degradation products in beer, stating that the isoelectric point of these substances lies between 4.6 and 5.5, and that they are very significant with respect to the properties of the beer. The coalescence of positively charged protein particles with negatively charged beer tannins is a well-known phenomenon. St. Johnston stated that his protein O is not affected by tannin because of the fact that the protein is negatively charged at the pH of wort and beer.

This and much more evidence is available to show that there are many nitrogenous colloids present in wort with isoelectric points between the pH of 6.0 and 3.9. As indicated by St. Johnston, mutual precipitation between some of these substances will undoubtedly take place in wort and will influence beer keeping properties.

Behavior of Caramels in Beer:

It is of interest to examine the behavior of some of

these colloids when the two types of caramel colorings described above are added to the beer. This will, of course, depend on the pH of the wort to which the caramel is added and changes will continually take place as the wort pH is lowered during fermentation. Assuming the wort p^H (or beer pH) to be 4.2 most of the proteins will have isoelectric points at about this figure and will thus be positively charged. Addition of a solution of caramel of isoelectric point pH 7.0 will introduce into the wort system a colloid having a strong positive charge. This should tend to be precipitated from solution.

Suppose, however, that a caramel of low isoelectric point at about pH 3.0 is used for beer coloring. It will be negatively charged (this type of coloring matter carries only a weak charge), and will thus tend to unstabilize and, ultimately, to precipitate some of the beer proteins and other colloids that are positively charged. Only the negatively charged colloids present, such as the tannin materials and possibly "protein O", will be stabilized. Natural coloring matter of melanoidin type will also enter into these various precipitation and stabilization systems depending upon their isoelectric points. The use of the wrong type of caramel coloring will, therefore, certainly tend to instability in the beer. It is significant that some caramel manufacturers now recommend only caramel of high isoelectric point for beer coloring.

It is of great importance to realize that the precipitation reactions just described often do not take place instantaneously. Owing to the very small amount of the electrical charge carried by some of the materials, and to the protective actions of the colloids present, it may well be that the precipitations will require days, or even weeks, to take place. This means that an incorrect caramel may cause the deposit of unpleasant hazes and precipitates in the bottle or can.

The mixing of solutions of high and low isoelectric point caramels at a pH intermediate between their isoelectric pH values results in the mutual precipitation of some of the color of the two caramels. The reason for the precipitation

was not understood until recently, and was ascribed to "incompatability" by Fetzer. (7). This author, however, wisely warned against the mixing together of two or more batches of caramel of differing origin.

In the coloring of soft drinks in which kola, bur-dock, and other herbs are employed, the use of high isoelec-tric point (positively charged) caramel results in the precipitation of the negatively charged tannin-like sub-stance, present in the natural plant materials. A caramel of low isoelectric point, negatively charged at the pH (3.5 or so) of the solution is used for these beverages and to stabilize the system and produce a beverage of good clar-ity. Breweries having an interest in this type of trade, even indirectly, are warned not to mix their caramel coloring materials.

It may be mentioned that when yeast is added to a wort a new electrical system is introduced. Yeast cells at these normal pH values are often negatively charged, and can there-fore adsorb positively charged colloids, including some of the caramel color. This is partly responsible for the color of compressed yeast since some natural melanoidins are unstable in solutions of low pH and may precipitate on, and be separa-ted off with the yeast.

It is interesting to note that some inferior caramel coloring substances available commercially have been prepared in such a manner that they precipitate when dissolved in water; or in water acidified tothe pH of beer. This occurs when un-controlled production during manufacture of the coloring matter results in particles large enough to precipitate at beer pH values. A good caramel should exhibit complete stab-ility in solution.

Literature:

1.   J. of the Inc. Brewers' Guild, Jan. 1947.

2.   J. Inst. Brew. 1948, 319.

3.   J. Inst. Brev. 1950, 232

4.   J. Inst. Brew. 1941, 47, 106.

5.   1st Int. Cong. Biochem. Abstracts 548.

6.   Brewing Science and Practice, London, 1938, 1,p.149

7.   Ind. Eng. Chem. Anal. Ed. 1933, 10, 349.

SPECIFICATION FOR

CARAMEL

FOR USE

IN FOODSTUFFS

B.S. 3874 : 1965

B.S. 3874 : 1965

This standard makes reference to the following British Standards:
B.S. 733. Density bottles.
B.S. 3210. Methods for the analysis of water-soluble coal-tar dyes permitted for use in foods.

CO-OPERATING ORGANIZATIONS

The Chemicals Industry Standards Committee, under whose supervision this British Standard was prepared, consists of representatives from the following Government departments and scientific and industrial organizations:

*Association of British Chemical Manufacturers
Board of Trade
British Iron and Steel Federation
Fertiliser Manufacturers' Association Ltd.
Gas Council
Institute of Vitreous Enamellers
Institution of Gas Engineers
Ministry of Defence, Army Department
*Ministry of Health
National Sulphuric Acid Association
Royal Institute of Public Health and Hygiene

The Government department and industrial organization marked with an asterisk in the above list, together with the following, were directly represented on the committee entrusted with the preparation of this British Standard:

Association of Public Analysts
British Baking Industries Research Association
British Colour Makers' Association
British Essence Manufacturers' Association
British Food Manufacturing Industries Research Association
British Industrial Biological Research Association Ltd.
British Pharmacopoeia Commission
Cake and Biscuit Alliance
Caramel Manufacturers
Cocoa, Chocolate and Confectionery Alliance
D.S.I.R.—Laboratory of the Government Chemist
Flavouring Compound Manufacturers' Association
Food Manufacturers Federation Incorporated
Ministry of Agriculture, Fisheries and Food
Ministry of Health and Local Government (Northern Ireland)
Pharmaceutical Society of Great Britain
Royal Society for the Promotion of Health
Society for Analytical Chemistry
Society of Chemical Industry (Food Group)
Society of Dyers and Colourists

3

B.S. 3874 : 1965

BRITISH STANDARD SPECIFICATION FOR

CARAMEL FOR USE IN FOODSTUFFS

FOREWORD

The British Standards Institution has been asked by the Ministry of Agriculture, Fisheries and Food to prepare specifications for the colouring matters permitted by the Colouring Matter in Food Regulations, 1957*, and this British Standard is one of a series covering materials, other than coal-tar dyes, listed in these Regulations.

In the preparation of these standards regard has been paid to the degree of purity commercially obtainable by good manufacturing practice, the needs of users and the requirements of overseas standards, particularly any relevant specifications approved by the Food and Drug Administration of the United States.

The term 'caramel' does not denote a single chemical substance. A wide range of types of caramel is made to suit the requirements of particular users. This British Standard, therefore, does not go beyond the specification of limits for harmful impurities, and of such characteristics as will ensure an acceptable standard of quality for users who do not agree detailed specifications with manufacturers or suppliers.

SPECIFICATION

SCOPE

1. This British Standard applies to caramel for use in the colouring of foodstuffs.

DEFINITION

2. Caramel is the water-soluble brown material prepared by the action of heat on water-soluble carbohydrates, or mixtures of such carbohydrates, in the presence or absence of acids or alkalis or of combinations of these.

RAW MATERIALS

3. Each material used in the manufacture of caramel shall individually comply with any appropriate statutory regulations or other relevant standards of purity for the particular material when it is to be used in the manufacture of foodstuffs.

* Statutory Instrument 1957 No. 1066, obtainable from H.M.S.O.

DENSITY OF 10 PER CENT SOLUTION

4. *a.* Unless otherwise agreed between purchaser and vendor, the density of a 10·00 per cent w/v solution of the caramel shall be not less than 1·0213 g/ml at 20°C when determined by the method described in Appendix A.

NOTE. This minimum value corresponds to a solids content of approximately 65 per cent w/w.

b. For the purposes of Clauses 6, 7 and 8 of this specification, the solids content of the caramel shall be calculated from the density of the 10 per cent solution by means of the formula given in Appendix A, Subclause A4*b*.

COLOUR INTENSITY

5. Unless otherwise agreed between purchaser and vendor, the colour intensity of the caramel shall be not less than 20 000 EBC units when determined by the method described in Appendix B.

COPPER

6. The caramel shall not contain more than 20 parts per million of copper, Cu, calculated on the dry basis and determined by the method described in B.S. 3210*.

ARSENIC

7. The caramel shall not contain more than 3 parts per million of arsenic, As, calculated on the dry basis and determined by the method described in B.S. 3210*.

LEAD

8. The caramel shall not contain more than 5 parts per million of lead, Pb, calculated on the dry basis and determined by the method described in B.S. 3210*.

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* B.S. 3210, 'Methods for the analysis of water-soluble coal-tar dyes permitted for use in foods'.

B.S. 3874 : 1965

APPENDIX A

METHOD FOR DETERMINATION OF DENSITY OF CARAMEL SOLUTION AND CALCULATION OF SOLIDS CONTENT

A1. Definition. For the purposes of this British Standard, the density of a liquid at $t°C$ is taken as the weight in air, in grammes, of one millilitre of the liquid at $t°C$ against weights adjusted to balance brass weights in air.

A2. Apparatus. Density bottle complying with B.S. 733*, of capacity 50 ml.

A3. Procedure. *a.* Weigh 10·00 g of the caramel, dissolve it in freshly boiled and cooled distilled water and dilute the solution to 100 ml at $20 \pm 0·1°C$ in a volumetric flask.

b. Clean and dry the bottle and weigh it to the nearest milligramme. Using freshly boiled and cooled distilled water at $20 \pm 0·1°C$, rinse the bottle several times and then fill it, inserting the stopper in such a way that the capillary is completely filled with water. Dry the outside of the stopper and bottle and weigh.

c. Empty the bottle, rinse it several times with the caramel solution at $20 \pm 0·1°C$ and then fill it with the solution. Insert the stopper as before, dry the outside of the stopper and bottle and weigh.

A4. Calculations. *a.* Calculate the density of the caramel solution from the formula

$$\text{Density} = \frac{0·9972\,W_2}{W_1} \text{ g/ml,}$$

where W_1 = weight, in grammes, of water, contained in bottle at $20°C$,

   W_2 = weight, in grammes, of caramel solution, contained in bottle at $20°C$,

and $0·9972$ = weight of 1 ml of freshly distilled water at $20°C$ against brass weights in air of density $0·0012$ g/ml.

b. Calculate the solids content of the caramel from the formula:

Solids content, per cent w/w = 2688 (Density *minus* 0·9972).

---

* B.S. 733, 'Density bottles'.

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APPENDIX B

METHOD FOR MEASUREMENT OF COLOUR INTENSITY*

B1. Principle. A solution of the caramel, of suitable known concentration, is compared visually with a permanent colour standard. The colour intensity is expressed in arbitrary units, being calculated from the designation of the matching colour slide and the concentration of the solution.

B2. Apparatus†.

   a. Colour comparator.

   b. Glass cell suitable for use with the comparator, of 25 mm thickness.

   c. Glass colour slide, 20° EBC.

NOTE. EBC slides are composed of superimposed Lovibond Red and Yellow slides. The value of the 20° slide is equal to 4R + 15Y where R and Y are the values of the Red and Yellow slides, respectively, on the Lovibond Scale.
   The chromaticity co-ordinates of the 20° slide on the C.I.E. system are:
$$x = 0.497; y = 0.443; z = 0.060.$$

   d. Source of artificial north daylight: Commission Internationale de l'Eclairage standard B (equivalent black body radiation temperature = 4800° K) at an intensity of 100–1000 lux (approximately 10–100 foot lamberts) and employing a white reflecting surface of over 95 per cent reflectivity.

B3. Procedure. *a.* Prepare a 0.100 per cent w/v solution of the caramel in freshly boiled and cooled distilled water. If the solution is not brilliant, clarify it by centrifuging, but *not* by filtration.

   b. Place the solution in the cell and compare it with the EBC slide. For the comparison use either diffuse north daylight or the source of artificial north daylight specified above.

NOTE 1. Caramel solutions often leave a very adherent deposit on the sides of the glass cells, usually invisible, but capable of affecting the colour reading obtained. This film is most readily removed by washing with a dilute solution of sodium carbonate or sodium hydroxide. It can also be removed by rubbing with cotton wool.
NOTE 2. Cells should be checked from time to time to make sure that when filled with distilled water they do not show any colour reading.

B4. Reporting of result. The 20° EBC slide, used in conjunction with a 0.100 per cent w/v solution of the caramel in a 25 mm cell, corresponds to a colour intensity of 20 000 EBC units.

   Report the colour intensity of the test solution as 'Not less than 20 000 EBC units' or as 'Less than 20 000 EBC units', as appropriate.

* This method is based on Method 304E of the European Brewery Convention (EBC).
† Suitable apparatus is obtainable from Tintometer Ltd., Salisbury, Wilts.

Scientific Associates

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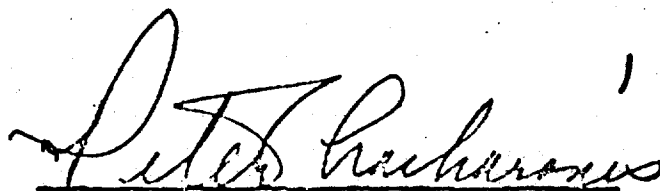
June 10, 1960

CERTIFICATE OF ANALYSIS

CHRONIC TOXICITY STUDY
CARAMEL COLORS A AND B

S.A. 54219

Peter Chacharonis, Ph.D.

TABLE OF CONTENTS

TABLES

INTRODUCTION

The object of this study was to establish adequate
safety of Caramel Colors A and B after repeated
ingestion. The study was designed to determine if
any ill effects or pathological changes are produced
in albino rats when large doses of caramel colors A
and B are admixed to the daily diet for a period of
three months.

TO: Union Starch and Refining Co.

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EXPERIMENTAL PROCEDURE:

The Animals - Sprague-Dawley albino rats were used in this study. Weanling rats were divided into groups of 20 animals each, ten of which were males and ten females.

Maintenance.- All animals were maintained in individual cages and closely observed for signs of ill effect or abnormal behavior. The rats were fed Purina Laboratory Chow which was given ad libitum. Weekly food consumption recorded(Tables IV, V, and VI). Water was given ad libitum.

Weights of all the animals were recorded weekly and are shown in Tables I, II, and III.

Dosage Levels - The caramel colors were admixed to ground Purina Laboratory Chow. The mixture was prepared weekly and the doses were recalculated using the average food consumption records.

The dosage levels were as follows:

> Group I - High Dose of Caramel Color A -10 gms/kilogram body weight.

> Group II - High Dose of Caramel Color B - 5gms/kilogram body weight.

> Group III - Control

Blood, Urine and Tissue Studies

Blood tests were made at the beginning of the study, and at monthly intervals thereafter. The tests were carried out using Schillings Classification. The results are shown in Tables VII, VIII and IX. The tests included the following:

> Red Blood Count
> Hemoglobin
> Hematocrit
> White Blood Count
> Differential Count

Urine analysis were performed at the beginning of the study and at monthly intervals thereafter. The results are shown in Tables X, XI and XII. The following were observed:

> Albumen
> Acetone
> Sugar

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At the end of the experimental period 10 animals from each group
were sacrificed and examined for signs of gross systemic damage.
The following organs were removed for micropathology:

Liver	Heart
Spleen	Gastrointestinal tract
Pancreas	Lymphoid Elements
Lungs	Adrenals
Kidneys	Thyroid
Brain	Parathyroid

The tissues were preserved in 10% neutral formalin and subsequently
imbedded, sectioned and stained with Hematoxylin and Eosin.

The microscopic examination of the organs was performed by
W. R. Platt, M.D., F.A.C.P., Pathologist.

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RESULTS AND DISCUSSION

Group I
High Dose of Caramel Color A - 10 grams/Kg.

The animals of this group showed normal weight gain(Table I).
Food consumption was within normal limits and compared favorably
with that of the control animals(Table IV).

The blood picture(Table VII) is normal and shows no significant
hematological alterations as compared to the control animals.

Urine analysis did not reveal significant abnormalities as
compared to the control group(Table X).

Autopsy revealed no gross systemic damage. The mucous layer of the
gastrointestinal tract showed some coloration of the caramel color
but this is not remarkable and is certainly to be expected at such
high dosage levels. Moreover, microscopic examination of the sections
of the intestinal tract did not disclose any degeneration changes whatsoever.

Microscopic description:

Rat No. 1 - Sections of brain, lung, liver, heart, thyroid, parathyroid,
kidney, adrenals and gastrointestinal tract show no significant microscopic
changes. There is no evidence of any infiltration of inflammatory cells, no
evidence of any specific degenerative process and no evidence of marked
congestion.

Rat No. 3 - Sections of spleen, skeletal muscle, attached cartilage and lung,
together with heart muscle, kidneys, pancreas and portions of the gastro-
intestinal tract show no significant microscopic changes.

Rat No. 5 - Sections of spleen, lung, liver, gastrointestinal tract,skeletal
muscle with focal cartilage, pancreas and portions of the large bowel and
kidney show no significant microscopic changes.

Rat No. 7 - Sections of the tissues from this animal show no evidence of any
inflammatory,degenerative or neoplastic process. These include sections of
the brain, lung, liver, heart, salivary gland structures, kidneys and
portions of the gastrointestinal tract.

Rat No. 9 - Sections of the brain, lung, liver, heart, salivary glands,kidneys,
adrenals, spleen and portion of the gastrointestinal tract show no significant
microscopic changes.

Rat No. 10 - Sections of the brain, including the Sommer's Sector, show no
evidence of degenerative, neoplastic or glial infiltration, no loss of
pyramidal cells. The lungs are not remarkable. Liver, heart, salivary gland
structures, spleen, kidneys and portions of the gastrointestinal tract show
no evidence of degenerative, neoplastic process.

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Group I - Cont.

Rat No. 13 - Sections of the spleen and kidney show marked interstitial congestion. The liver shows some congestion around the portal triad areas. The rest of the sections from the brain, gastrointestinal tract, pancreas, heart, lung and brain, including Sommer's area, show no evidence of any microscopic histologic changes.

Rat No. 15 - Sections of the brain, lungs, liver, myocardium, spleen, kidney and portions of gastrointestinal tract show some slight interstitial renal congestion, otherwise not remarkable.

Rat No. 17 - Sections of pancreas, kidneys, gastrointestinal tract, myocardium, liver, lungs and brain and portions of the spleen show some slight congestion of the splenic sinusoids, otherwise not remarkable.

Rat No. 19 - Sections of the lungs show lymphoid aggregates around the larger branches of the bronchi and bronchioles and in the subpleural area with moderate to marked interstitial congestion. The alveolar spaces are not remarkable. Sections of the brain, liver, myocardium, pancreas, salivary glands, kidneys and gastrointestinal tract show no other significant microscopic changes.

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Group II

High Dose of Caramel Color B - 5 grams/Kg.

The animals of this group showed normal weight gain(Table II).
Food consumption was within normal limits and compared favorably
with that of the control animals(Table V).

The blood picture(Table WIII) is normal and shows no significant
hematological alterations as compared to the control animals.

Urine analysis did not reveal significant abnormalities as compared
to the control group(Table XI).

Autopsy revealed no gross systemic damage. The mucous layer of the
gastrointestinal tract showed some coloration of the caramel color but
this is not remarkable and is certainly to be expected at such high
dosage levels. Moreover, microscopic examination of the sections of
the intestinal tract did not disclose any degeneration changes whatsoever.

Microscopic description:

Rat No. 1 - Sections of brain, lung, kidneys, gastrointestinal tract,
myocardium, liver and salivary gland structures show no evidence of
any microscopic alteration.

Rat No. 3 - Sections of kidney show moderate interstitial congestion.
Section of brain and Sommer's Sector area, lymph nodes, spleen, gastro-
intestinal tract, liver, lungs and salivary gland structures show no
significant microscopic changes.

Rat No. 5 - Sections of brain, lung, liver, heart, salivary gland structures,
kidneys, lymph nodes, spleen and gastrointestinal tract show no significant
microscopic changes.

Rat No. 7 - Sections of brain, lung, liver, heart, salivary glands, kidneys,
spleen, lymph nodes and gastrointestinal tract show no microscopic alteration.

Rat No. 10 - Sections of brain, lung, liver, myocardium, salivary glands,
pancreas, kidneys, lymph nodes, spleen and gastrointestinal tract show no
changes except in the renal pelvic-cortico-medullary junction there
appears to be some interstitial congestion.

Rat No. 11 - Sections of brain, liver, myocardium, lung, salivary glands,
pancreas, kidneys, and gastrointestinal tract show no significant micro-
scopic changes.

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Rat No. 13 - Sections of brain, lung, liver, heart, salivary glands, kidneys, spleen, lymph nodes and gastrointestinal tract show no significant microscopic alteration.

Rat No. 15 - Sections of lung show minimal interstitial inflammatory reaction and some congestion, otherwise not remarkable. Sections of the liver, brain, myocardium, salivary glands, kidneys and gastrointestinal tract show no significant changes.

Rat No. 17 - Sections of brain, lung, liver, myocardium, salivary glands, kidney, spleen, lymph node and gastrointestinal tract show no microscopic alteration.

Rat No. 19 - Sections of skin show intact epidermis underlying corium, otherwise not remarkable. Sections of skeletal muscle show focal areas of calcification as if this could be portion of a bony muscular insertion. The rest of the microscopic pattern of the brain, lungs, liver, myocardium, salivary glands, kidneys and gastrointestinal tract are not remarkable.

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Group III

Control

The animals of this group showed normal weight gains(Table III).
Food consumption was within normal limits(Table VI).

The blood picture(Table IX) is within normal limits.

Urine analysis(Table XII) did not reveal significant abnormalities
with the exception of the occasional appearance of minimal traces
of albumin and acetone which are considered insignificant and
within the limits of the experiment.

Autopsy revealed no gross systemic damage.

Microscopic description:

Rat No. 1 - Sections of kidney showe marked interstitial congestion.
This could be due to the terminal sacrificial ether administration.
The rest of the structures - myocardium, liver, etc. - show no
microscopic alteration.

Rat No. 3 - Sections of brain, lung, liver, heart, salivary glands,
kidneys, gastrointestinal tract and skin show no significant microscopic
changes.

Rat No. 5 - No alterations of microscopic pattern in any organs examined.

Rat No. 7 - Sections of brain, lung, liver, heart, salivary glands, spleen,
lymph nodes and gastrointestinal tract show no significant changes.

Rat No. 9 - Section of kidneys show moderate interstitial congestion.
Section of the heart, liver, lung, brain and gastrointestinal tract show
no significant microscopic changes.

Rat No. 11 - Sections of kidneys show interstitial congestion. Sections of
the lungs show some thickening of the alveolar wall. The rest of the organs
including gastrointestinal tract, myocardium, liver and brain show no
significant microscopic changes.

Rat No. 13 - Sections of spleen, salivary glands, skin and lymph nodes show
congestion, otherwise not remarkable. Sections of the brain, lung, myocardium
and gastrointestinal tract are not remarkable.

Rat No. 15 - Sections of the kidneys, gastrointestinal tract, salivary glands,
spleen, lymph nodes, myocardium, brain, lung and liver show no significant
microscopic changes.

Rat No. 17 - Sections of kidneys show interstitial congestion. Sections of liver,
myocardium, lung, brain, salivary gland, spleen, lymph nodes and gastro-
intestinal tract are not remarkable.

Rat No. 19 - Sections of brain, lung, liver, myocardium, salivary glands, spleen,
lymph nodes, kidneys and gastrointestinal tract show no significant microscopic
alteration.

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CONCLUSIONS AND SUMMARY:

The weight changes and food consumption of rats receiving high doses
of Caramel Colors A and B are within normal limits and do not show
significant differences from those of the control animals.

Evaluation of the overall blood picture(TableXIII) shows it to be
within normal limits and reveals no significant hematological changes
in rats given high doses of caramel colors A and B.

Urine analysis did not reveal significant abnormalities in the rats
receiving caramel colors A and B and was comparable to that of the
control animals.

Sections of the tissues from animals treated with Caramel Colors A and B
showed no significant microscopic alterations in any of the organs examined
as compared to those of the control group.

TABLE I

RAT WEIGHT RECORDS
(Weight in Grams)
Group I – High Dose of Sample A(10 grams/kilogram)

Rat No.	Sex	Initial	1	2	3	4	5	6	7	8	9	10	11	12	13	Total Wt. Change	% Total Weight Change
	F	55	85	140	175	190	200	220	200	225	240	250	250	250	260	+ 205	+ 372.72
	F	60	75	130	160	170	185	200	210	210	210	220	225	230	250	+ 190	+ 316.66
	F	70	80	140	160	175	175	200	200	200	210	220	210	210	230	+ 160	+ 228.57
	F	50	70	115	140	150	165	180	190	195	210	210	205	210	220	+ 170	+ 340.00
	F	65	90	140	180	190	205	215	230	240	240	220	250	250	260	+ 195	+ 300.00
	F	60	85	140	170	185	205	220	235	240	250	255	265	260	270	+ 210	+ 350.00
	F	65	85	140	165	180	195	210	210	230	250	255	250	255	265	+ 200	+ 307.69
	F	50	60	110	110	135	140	150	150	160	190	175	185	195	190	+ 140	+ 280.00
	F	55	80	120	140	150	165	175	180	175	190	190	200	200	205	+ 150	+ 272.72
	F	65	85	130	160	160	165	190	180	200	210	215	210	215	235	+ 170	+ 261.53
AVERAGE		59.5	79.5	130.5	156.0	168.5	190.0	196.0	198.5	207.5	220	221	225	227.5	238.5	+ 179.0	+ 302.98
	M	65	95	160	170	230	270	250	250	265	305	320	350	370	380	+ 315.0	+ 484.61
	M	80	105	170	165	230	270	275	290	300	330	350	360	365	375	+ 295	+ 368.75
	M	75	100	165	170	250	285	380	290	300	340	360	390	415	430	+ 355	+ 473.33
	M	65	90	150	180	240	260	290	290	300	320	335	365	390	405	+ 340	+ 523.07
	M	55	75	120	150	185	220	240	250	260	290	295	300	310	320	+ 265	+ 481.81
	M	60	85	140	130	190	210	240	250	260	300	310	340	355	375	+ 315	+ 525.00
	M	75	100	160	175	250	270	285	300	310	350	355	370	380	400	+ 325	+ 433.33
	M	65	80	150	155	225	255	270	280	280	315	320	350	360	380	+ 315	+ 484.61
	M	65	95	150	160	225	255	280	285	290	310	320	340	350	360	+ 295	+ 453.84
	M	75	100	150	175	230	250	265	270	275	315	330	320	340	350	+ 275	+ 366.66
AVERAGE		68.0	92.5	151.5	163.0	225.5	254.5	267.5	275.5	284.0	317.5	329.5	348.5	363.5	377.5	+ 309.5	+ 459.50
Male and Female		63.75	86	141.0	159.5	197.0	222.25	231.75	237.0	245.75	268.75	275.25	286.75	295.50	308	+ 244.25	+ 381.24

TABLE II

RAT WEIGHT RECORDS
(Weight in Grams)

GROUP II - High Dose of Sample B(5 grams/Kg)

WEEKS

Rat No.	Sex	Initial	1	2	3	4	5	6	7	8	9	10	11	12	13	Total Wt.Change	% Total Weight Change
1	F	60	85	130	150	160	170	185	190	200	210	220	240	240	260	+ 200	+ 333.33
2	F	55	75	140	145	190	200	220	230	225	235	240	250	250	260	+ 205	+ 372.72
3	F	65	80	140	160	175	190	200	215	225	240	240	245	250	260	+ 195	+ 300.00
4	F	55	75	120	140	175	200	230	240	260	265	270	275	280	290	+ 235	+ 427.27
5	F	65	90	145	160	180	190	190	210	210	240	240	240	250	250	+ 185	+ 284.61
6	F	70	85	135	150	160	185	210	220	220	235	250	245	250	250	+ 180	+ 257.14
7	F	55	85	140	170	190	200	220	230	220	240	245	250	250	260	+ 205	+ 372.72
8	F	45	65	90	115	140	150	180	185	195	185	190	210	250	250	+ 205	+ 455.55
9	F	60	80	120	150	155	165	185	195	190	195	210	215	220	220	+ 160	+ 266.66
10	F	70	95	140	130	170	180	200	210	210	230	235	230	245	240	+ 170	+ 242.85
AVERAGE		60	81.5	130	147.0	169.5	183	202	212.5	215.5	227.5	234	240	248.5	254.0	+ 194	+ 331.28
11	M	75	95	170	150	180	230	250	265	290	320	345	355	370	340	+ 265	+ 353.33
12	M	80	105	170	170	220	245	250	260	285	310	330	360	370	380	+ 300	+ 375.00
13	M	80	100	160	190	210	250	260	270	260	300	335	345	360	375	+ 295	+ 368.75
14	M	70	90	170	175	210	235	240	260	270	310	335	345	350	390	+ 320	+ 457.14
15	M	60	75	150	160	185	220	220	230	250	290	310	330	360	370	+ 310	+ 516.65
16	M	65	85	120	150	200	225	275	280	295	300	305	320	345	375	+ 310	+ 476.92
17	M	70	100	165	180	200	245	240	240	245	270	300	360	370	360	+ 290	+ 414.28
18	M	75	100	140	190	220	225	225	235	240	290	300	310	330	360	+ 285	+ 380.00
19	M	65	95	170	185	220	240	230	245	245	290	315	330	345	370	+ 305	+ 469.23
20	M	75	100	170	190	195	245	240	245	260	290	300	330	350	380	+ 305	+ 406.66
AVERAGE		71.5	94.5	158.5	174.0	204.0	236	243	253	264	297	317.5	338.5	355.0	370.0	+ 298.5	+ 427.79
Av. Male and Female		65.75	88.0	144.25	160.5	186.75	209.5	217.75	222.5	239.75	262.25	275.75	289.25	301.75	312.00	+ 246.25	+ 380.53

TABLE III

RAT WEIGHT RECORDS
(Weight in Grams)

GROUP III - CONTROL

Rat No.	Sex	Initial	WEEKS														Total Wt Change	% Total Wt Change
			1	2	3	4	5	6	7	8	9	10	11	12	13		
1	F	65	85	140	170	190	200	200	230	240	250	250	260	260	260	+ 195	+ 300.00
2	F	65	75	120	140	150	160	175	190	195	210	200	210	210	220	+ 150	+ 230.76
3	F	60	80	120	155	180	190	210	230	240	250	240	250	240	250	+ 190	+ 316.67
4	F	55	70	120	160	160	170	195	205	210	220	220	235	240	240	+ 185	+ 336.36
5	F	60	90	140	160	180	180	195	210	210	230	235	230	250	230	+ 170	+ 283.33
6	F	65	85	140	160	190	200	210	230	230	245	245	260	260	260	+ 195	+ 300.00
7	F	65	85	160	180	220	220	240	255	260	260	280	285	300	305	+ 240	+ 369.23
8	F	45	75	100	120	130	140	150	160	160	175	170	180	190	190	+ 145	+ 322.22
9	F	50	70	110	150	170	190	215	230	240	265	270	275	270	280	+ 230	+ 460.00
10	F	55	90	150	160	200	205	225	240	240	250	250	255	270	265	+ 215	+ 390.90
AVERAGE		58.5	80.5	130.0	155.5	177.0	185.5	201.5	218.0	222.5	235.5	236.0	244.0	249.0	250	+ 191.5	+ 330.94
11	M	60	95	170	170	235	235	270	250	320	305	340	360	370	390	+ 330	+ 550.00
12	M	75	105	190	190	280	320	325	345	350	390	400	430	450	440	+ 365	+ 486.66
13	M	80	100	175	170	250	280	305	350	320	360	380	390	410	420	+ 340	+ 425.50
14	M	70	100	180	180	260	300	320	320	320	380	380	415	425	430	+ 360	+ 514.28
15	M	55	60	80	100	115	130	140	170	195	220	235	240	230	250	+ 195	+ 354.54
16	M	60	80	150	195	240	270	290	230	310	340	340	350	370	380	+ 320	+ 533.33
17	M	75	100	170	210	250	265	290	285	300	340	350	350	360	370	+ 295	+ 393.33
18	M	65	90	165	180	230	260	300	320	310	350	350	385	400	405	+ 340	+ 523.07
19	M	60	80	160	210	230	260	275	300	305	320	335	335	350	360	+ 300	+ 500.00
20	M	75	100	150	185	230	250	270	280	290	310	335	340	340	350	+ 275	+ 366.66
AVERAGE		67.5	91.0	159.0	179.0	232.0	257.0	278.5	290.0	302.0	331.5	344.5	359.5	370.5	379.5	+ 312.0	+ 464.73
Av. Male and Female		63.00	85.75	144.50	167.25	204.5	221.25	240.0	254.0	262.25	283.5	290.25	301.75	309.75	314.75	+ 251.75	+ 397.83

TABLE IV
FOOD CONSUMPTION IN GRAMS
GROUP I - High Dose of Sample A(10 grams/kilogram)

WEEKS

Rat No.	Sex	1	2	3	4	5	6	7	8	9	10	11	12	13	Total Food Con.	Av. Wkly Fo. Cons.
1	F	135	155	110	130	120	115	115	105	140	100	115	135	105	1580	121.53
2	F	145	145	100	140	125	135	130	125	105	105	100	115	120	1590	122.30
3	F	135	155	135	135	130	135	125	130	135	135	100	125	110	1685	129.61
4	F	120	115	115	125	110	115	125	120	125	100	155	155	155	1635	125.76
5	F	145	145	100	120	140	135	140	110	120	125	145	100	130	1655	127.30
6	F	145	150	135	150	155	155	130	125	140	155	135	135	125	1815	139.61
7	F	135	150	100	110	150	155	155	135	145	100	135	125	135	1730	133.07
8	F	120	155	95	110	105	95	100	100	105	95	105	95	120	1400	107.69
9	F	120	145	135	125	115	105	130	115	120	100	95	115	125	1545	118.84
10	F	135	155	95	135	140	145	155	140	150	140	155	155	155	1855	142.69
Average		133.5	147.0	112.0	128.0	129.0	129.0	130.5	120.5	128.5	113.5	124.0	125.5	128.0	1649	126.84
11	M	150	155	155	160	140	135	150	135	160	155	190	180	190	2055	158.07
12	M	135	150	140	155	155	155	155	140	160	155	175	160	210	2045	157.30
13	M	155	165	140	150	155	160	135	155	170	155	220	205	215	2180	167.69
14	M	155	150	125	150	160	140	150	155	160	155	180	180	240	2100	161.53
15	M	145	155	155	155	145	135	155	140	150	120	110	160	150	1875	144.23
16	M	150	155	150	160	140	155	160	155	160	155	155	200	240	2135	164.23
17	M	140	145	155	145	150	155	150	155	160	130	130	150	160	1925	148.07
18	M	130	155	155	150	160	145	150	165	160	155	210	180	240	2135	155.76
19	M	125	140	150	140	155	155	140	155	165	140	195	170	190	2020	155.38
20	M	140	155	145	155	160	140	155	155	160	155	170	210	225	2125	163.46
Average		142.5	152.5	147.0	152.0	152.0	147.5	150.0	151.0	160.5	147.5	173.5	179.5	206.0	2061.5	158.57
Av. Male and Female		138.00	149.75	129.5	140.00	140.50	138.25	140.25	135.75	144.50	130.50	148.75	152.50	167.0	1855.25	142.70

TABLE V
FOOD CONSUMPTION IN GRAMS
GROUP II - High Dose of Sample B(5mg/Kg.)

WEEKS

Rat No.	Sex	1	2	3	4	5	6	7	8	9	10	11	12	13	Total Food Cons.	Av.Wkl Food Consun
1	F	115	105	85	120	140	140	125	115	120	120	105	105	100	1495	115.00
2	F	135	155	120	130	150	125	135	135	140	100	125	135	155	1740	133.84
3	F	105	155	100	120	130	120	140	145	155	100	145	120	150	1685	129.61
4	F	100	135	105	140	160	155	155	155	155	105	125	135	125	1750	134.61
5	F	125	155	105	155	65	155	155	155	155	100	135	140	155	1855	142.69
6	F	110	155	110	130	115	120	145	150	140	135	100	120	130	1660	127.69
7	F	125	155	115	145	165	145	140	155	155	105	120	115	125	1765	135.76
8	F	85	135	100	130	160	120	120	125	140	110	130	120	130	1605	123.46
9	F	120	140	110	120	150	120	130	155	155	100	115	110	155	1680	129.23
10	F	130	155	120	130	150	135	155	155	155	115	120	120	135	1775	136.53
Average		115.0	144.5	107.0	132.0	148.5	133.5	140.0	144.5	147.0	109.0	122.0	122.0	136	1701.0	130.84
11	M	155	130	155	140	135	155	160	135	165	150	190	200	190	2060	158.46
12	M	155	120	145	135	140	160	150	140	160	155	220	180	230	2090	160.76
13	M	130	150	140	145	155	150	150	145	165	150	205	170	185	2040	156.92
14	M	150	145	155	150	155	155	145	155	170	160	180	190	243	2150	165.38
15	M	150	155	135	150	145	160	155	160	160	150	210	190	200	2120	163.07
16	M	135	150	140	155	150	155	160	155	160	145	210	195	240	2150	165.38
17	M	155	140	150	145	155	160	145	165	160	155	230	200	140	2150	165.38
18	M	125	150	155	145	145	150	155	155	165	160	180	180	200	2065	158.84
19	M	155	140	150	155	145	155	150	155	170	150	190	210	215	2140	164.61
20	M	155	135	140	155	150	160	155	160	160	155	170	220	240	2145	165.00
Average		146.5	141.5	146.5	147.5	147.5	156.0	152.5	151.5	163.5	153.0	198.5	193.5	213.0	2111.0	162.38
Av.Male and		130.75	143.0	126.75	139.75	148.0	144.75	146.25	148.0	155.25	131.0	160.25	157.75	174.5	1906.0	146.61

TABLE VI
FOOD CONSUMPTION IN GRAMS
GROUP III - CONTROL

WEEKS

Rat No.	Sex	1	2	3	4	5	6	7	8	9	10	11	12	13	Total Food Cons.	Av.Wkly Food Consum
1	F	95	130	85	100	120	105	105	125	135	120	135	125	95	1475	113.46
2	F	90	120	85	105	115	95	100	100	100	195	110	125	155	1495	115.00
3	F	95	140	80	135	145	105	125	100	105	100	125	150	115	1520	116.92
4	F	80	135	80	110	110	105	125	115	140	110	125	115	155	1505	115.76
5	F	80	130	90	130	150	125	130	120	135	105	135	115	125	1570	120.76
6	F	105	155	90	100	120	100	115	105	100	105	125	145	145	1510	116.15
7	F	115	125	105	105	115	125	125	125	130	125	130	95	120	1540	118.46
8	F	80	100	60	90	110	95	95	100	100	95	100	125	130	1280	98.46
9	F	75	100	115	120	130	125	120	120	130	110	135	125	115	1520	116.92
10	F	105	155	115	120	110	105	155	110	120	100	105	135	135	1570	120.76
Average		92.0	129.0	90.5	111.5	122.5	108.5	119.5	112.0	119.5	116.5	122.5	125.5	129.0	1498.5	115.26
11	M	125	150	125	150	160	150	155	180	155	155	200	195	195	2095	161.15
12	M	135	155	155	155	145	155	160	155	195	155	210	210	200	2135	168.07
13	M	125	155	155	155	150	160	150	155	175	160	190	195	190	2115	162.69
14	M	120	155	155	140	155	155	155	155	195	150	195	215	240	2185	168.07
15	M	55	85	65	90	105	100	100	125	155	120	130	140	155	1425	109.61
16	M	110	125	150	140	135	150	145	155	175	160	240	210	240	2135	164.23
17	M	125	140	120	130	145	155	135	150	180	155	190	190	195	1990	153.07
18	M	125	155	155	150	150	165	160	145	170	155	190	220	240	2180	167.69
19	M	115	145	120	145	150	140	165	150	165	155	175	185	200	2010	154.61
20	M	125	155	160	155	155	150	155	155	170	150	185	190	240	2145	165.00
Average		116.0	142.0	136.0	141.0	145.0	148.0	148.0	152.5	173.5	151.5	188.5	195.0	209.5	2046.5	157.42
Av. Male and Female		104.0	135.50	113.25	126.25	133.75	128.25	133.75	132.25	146.50	134.0	155.5	160.25	169.25	1772.5	136.34

TABLE VII

BLOOD PICTURE - RATS

GROUP I - High Dose of Sample A (10 grams/kilogram)

Grp and Dose level	Rat No.	Sex		RBC in millions	Hemoglobin (gms per 100 cc.)	Hemato-crit vol %	WBC	Eosino philes	Baso philes	Myelo cytes	Juven iles	Stab	Segmen-ted	Lympho cytes	Mono cyt
Group I A	1	F	Initial	8.0	16.6	56	11,500	1	-	-	-	-	19	78	1
			1 month	7.7	15.8	51	12,000	3	-	-	-	-	18	79	3
			3 months	8.1	17.0	46	11,400	4	-	-	-	1	20	72	4
	2	F	Initial	7.8	17.2	50	14,500	1	-	-	-	-	53	42	1
			1 month	8.2	18.0	54	13,000	2	-	-	-	2	14	84	-
			2 months	8.0	16.0	56	16,000	-	-	-	-	-	35	64	-
			3 months	8.4	18.2	51	15,200	1	-	-	-	-	20	53	2
	3	F	Initial	8.6	16.6	43	15,000	1	-	-	-	-	39	57	1
			1 month	8.7	18.4	52	11,300	7	-	-	-	-	27	66	7
			2 months	8.4	16.8	51	10,450	4	-	-	-	-	16	78	4
			3 months	8.5	16.0	50	8,400	1	-	-	-	-	30	66	3
	4	F	Initial	6.5	13.6	43	16,300	1	-	-	-	1	23	71	1
			1 month	7.7	18.0	50	12,700	12	-	-	-	1	36	51	12
			2 months	7.7	17.6	50	12,650	5	-	-	-	-	25	70	5
			3 months	9.8	17.0	53	14,150	7	-	-	-	-	25	67	1
	5	F	Initial	6.8	14.4	43	12,100	-	-	-	-	-	29	69	-
			1 month	7.9	16.8	50	12,050	11	-	-	-	1	23	65	11
			2 months	7.7	16.2	46	15,000	2	-	-	-	2	36	60	2
			3 months	7.8	17.4	51	18,300	1	-	-	-	-	28	70	1
	6	F	Initial	8.1	15.5	51	19,800	1	-	-	-	3	20	74	1
			1 month	7.3	18.2	50	17,700	7	-	-	-	-	32	61	7
			2 months	8.5	17.5	54	14,400	4	-	-	-	1	29	64	4
			3 months	8.1	18.8	50	19,400	2	-	-	-	-	29	67	2
	7	F	Initial	7.8	16.8	46	15,400	-	-	-	-	4	25	60	-
			1 month	7.8	16.8	46	14,300	2	-	-	-	-	18	80	2
			2 months	8.7	17.4	47	19,200	1	-	-	-	-	28	91	1
			3 months	9.0	17.0	47	15,700	-	-	-	-	-	17	82	1
	8	F	Initial	8.1	17.8	45	13,700	-	-	-	-	2	19	75	-
			1 month	8.9	19.6	58	15,800	3	-	-	-	-	44	53	3
			2 months	7.5	18.8	52	16,000	-	-	-	-	-	38	59	5
			3 months	8.6	19.4	55	17,050	3	-	-	-	-	28	64	

TABLE VII(Cont.)
BLOOD PICTURE - RATS
Group I - High Dose of Sample A

Grp and Dose Level	Rat No.	Sex		RBC in millions	Hemoglobin (gms per 100 cc)	Hematocrit vol %	WBC	Eosino philes	Baso philes	Myelo cytes	Juve niles	Stab	Segmen ted	Lympho cytes	Mono cytes
Grp I A	9	F	Initial	7.5	16.4	49	15,200	-	-	-	-	3	25	66	-
			1 month	7.8	17.6	54	14,800	1	-	-	-	-	35	64	1
			2 months	8.5	17.5	51	14,400	4	-	-	-	-	38	57	4
			3 months	8.5	17.8	46	12,300	2	-	-	-	-	32	59	6
	10	F	Initial	7.2	17.0	43	13,200	2	-	-	-	-	26	70	2
			1 month	8.6	17.2	51	9,950	9	-	-	-	-	36	55	-
			2 months	7.5	16.2	46	13,200	2	-	-	-	-	48	48	2
			3 months	8.1	16.1	51	12,400	5	-	-	-	1	57	33	4
	11	M	Initial	7.6	17.4	46	11,600	-	-	-	-	-	41	53	4
			1 month	8.3	17.2	50	19,300	1	-	-	-	-	21	78	-
			2 months	9.0	15.4	51	17,000	2	-	-	-	-	34	59	5
			3 months	7.7	15.0	52	12,500	3	-	-	-	-	26	69	2
	12	M	Initial	6.8	16.0	47	17,000	4	-	-	-	2	43	45	6
			1 month	7.8	16.2	47	20,000	4	-	-	-	-	16	79	1
			2 months	8.8	15.6	49	13,000	-	-	-	-	-	14	86	-
			3 months	7.7	14.8	50	16,700	1	-	-	-	-	21	77	1
	13	M	Initial	7.2	17.0	50	17,900	-	-	-	-	3	47	45	5
			1 month	7.4	16.8	50	21,000	3	-	-	-	-	20	77	-
			2 months	8.6	16.3	52	13,600	1	-	-	-	2	18	78	1
			3 months	7.4	15.1	46	20,400	6	-	-	-	-	21	72	1
	14	M	Initial	7.0	16.9	46	14,600	-	-	-	-	-	20	71	-
			1 month	7.9	16.2	51	18,000	1	-	-	-	-	16	81	2
			2 months	7.2	16.8	51	14,200	-	-	-	-	-	15	81	4
			3 months	811	16.2	49	16,200	1	-	-	-	-	10	88	1
	15	M	Initial	7.7	16.0	54	9,900	-	-	-	-	1	14	83	2
			1 month	6.5	16.6	53	16,500	7	-	-	-	1	19	59	4
			2 months	7.8	17.8	51	18,000	3	-	-	-	-	31	65	1
			3 months	7.3	18.2	48	15,700	1	-	-	-	-	11	86	2
	16	M	Initial	9.9	15.4	39	14,300	-	-	-	-	1	15	79	5
			1 month	8.4	17.0	49	11,650	2	-	-	-	-	18	79	1
			2 months	8.7	17.8	52	14,800	1	-	-	-	-	8	91	-
			3 months	7.1	15.4	50	13,500	4	-	-	-	-	16	80	-
	17	M	Initial	6.8	13.8	42	11,400	1	-	-	-	-	55	43	1
			1 month	7.9	15.2	54	17,300	3	-	-	-	-	22	71	4
			2 months	8.3	15.2	50	15,800	2	-	-	-	-	27	69	2
			3 months	7.7	14.3	50	15,700	3	-	-	-	1	23	23	

TABLE VII(Cont)
BLOOD PICTURE - RATS

GROUP I & High Dose of Sample A(10 grams/kilogram)
Differential Count

Grp and Dose Level	Rat No.	Sex		RBC in millions	Hemoglobin (gms per 100 cc)	Hemato-crit vol %	WBC	Eosino philes	Baso philes	Myelo cytes	Juven iles	Stab	Segmen ted	Lympho cytes	Mono cyt
Grp I	18	M	Initial	6.8	15.8	48	11,600	-	-	-	-		32	64	3
A			1 month	8.4	17.4	47	15,000	-	-	-	-	1	20	78	1
			2 months	8.5	16.5	52	17,000	2	-	-	-	1	33	60	5
			3 months	7.7	15.4	50	13,650	-	-	-	-	-	30	65	5
	19	M	Initial	7.3	16.2	48	17,600	-	-	-	-	-	36	63	1
			1 month	6.7	17.2	47	12,000	3	-	-	-	2	25	69	1
			2 months	8.6	16.3	52	17,000	2	-	-	-	-	16	81	1
			3 months	8.6	16.7	51	13,400	1	-	-	-	-	20	79	-
	20	M	Initial	6.2	15.0	42	11,100	-	-	-	-	-	50	49	1
			1 month	9.3	17.0	59	13,000	1	-	-	-	-	12	87	-
			2 months	7.7	16.2	53	20,850	-	-	-	-	-	8	92	-
			3 months	7.9	15.8	51	14,500	2	-	-	-	-	28	68	2

TABLE VIII
BLOOD PICTURE - RATS
GROUP II - High Dose of Sample B(5 mg/Kg)

Grp and Dose level	Rat No.	Sex		RBC in millions	Hemoglobin (gms per 100 cc)	Hematocrit vol %	WBC	Differential Count Eosinophiles	Baso philes	Myelo cytes	Juven iles	Stab	Segmen ted	Lympho cytes	Mono cyt
Grp II B	1	F	Initial	7.4	14.6	42	17,300	—	—	—	—	—	18	8	1
			1 month	7.2	15.8	48	12,250	4	—	—	—	z.	37	58	1
			2 months	8.4	15.4	52	18,500	2	—	—	—	—	34	60	1
			3 months	6.8	35.0	49	16,900	—	—	—	—	—	16	82	4
	2	F	Initial	6.1	14.6	40	12,600	—	—	—	—	—	16	82	2
			1 month	7.8	15.8	47	11,150	1	—	—	—	—	20	77	3
			2 months	8.0	16.0	49	17,050	—	—	—	—	—	25	72	2
			3 months	8.0	15.8	46	11,200	—	—	—	—	—	10	89	1
	3	F	Initial	7.2	17.8	40	19,400	—	—	—	—	—	15	83	2
			1 month	7.7	15.7	48	16,000	6	—	—	—	1	33	66	—
			2 months	7.9	15.8	48	12,800	—	—	—	—	—	20	72	2
			3 months	7.3	17.2	48	15,550	5	—	—	—	—	26	74	3
	4	F	Initial	5.9	16.8	44	20,400	5	—	—	—	—	40	52	3
			1 month	6.0	15.2	44	19,600	2	—	—	—	—	13	86	1
			2 months	9.0	16.2	51	18,850	2	—	—	—	—	4	94	—
			3 months	8.3	15.2	47	20,450	3	—	—	—	—	23	72	3
	5	F	Initial	7.5	14.2	42	14,500	1	—	—	—	1	21	74	1
			1 month	6.7	14.0	47	19,700	12	—	—	—	3	32	64	1
			2 months	7.8	14.0	49	15,750	1	—	—	—	—	39	48	1
			3 months	9.2	14.4	50	11,150	—	—	—	—	—	41	58	—
	6	F	Initial	6.6	13.4	39	8,600	—	—	\	—	1	14	85	—
			1 month	7.0	14.6	42	10,400	1	—	—	—	z.	23	77	—
			2 months	7.4	15.1	44	9,900	—	—	—	—	—	29	61	2
			3 months	8.6	14.6	49	11,150	2	—	—	—	—	33	72	1
	7	F	Initial	6.3	18.1	42	13,400	—	—	—	—	1	41	53	3
			1 month	7.8	16.5	48	19,700	—	—	—	—	2	32	64	2
			2 months	8.7	17.6	50	11,550	—	—	—	—	—	34	64	2
			3 months	8.8	16.4	45	13,400	5	—	—	—	—	17	74	4
	8	F	Initial	6.9	17.6	50	15,300	—	—	—	—	—	23	71	5
			1 month	8.2	18.2	54	19,500	7	—	—	—	—	27	55	—
			2 months	8.7	16.6	55	17,950	4	—	—	—	2	23	68	—
			3 months	8.2	17.3	52	15,100	9	—	—	—	—	24	68	4
	9	F	Initial	8.0	18.2	48	11,250	—	—	—	—	—	11	75	5
			1 month	8.4	16.8	48	12,000	3	—	—	—	1	69	30	—
			2 months	9.5	15.3	52	19,000	3	—	—	—	—	19	75	3

TABLE VIII(Cont)
BLOOD PICTURE - RATS

Grp and Dose	Rat No.	Sex		RBC in millions	Hemoglobin (gms per 100 cc)	Hematocrit vol %	WBC	Eosinophiles	Basophiles	Myelocytes	Juveniles	Stab	Segmented	Lymphocytes	Monocytes
Grp II B	10	F	Initial	6.0	15.4	36	18,200	-	-	-	-	2	32	66	-
			1 month	8.8	16.2	49	17,000	4	-	-	-	-	25	71	-
			2 months	8.6	15.0	46	17,500	1	-	-	-	-	12	85	2
			3 months	8.2	16.0	48	15,700	-	-	-	-	-	18	73	2
	11	M	Initial	8.0	15.6	44	19,300	-	-	-	-	2	42	54	1
			1 month	8.4	16.2	52	13,000	2	-	-	-	-	27	71	-
			2 months	8.7	16.2	52	14,000	3	-	-	-	-	25	72	-
			3 months	6.8	15.0	49	12,300	-	-	-	-	-	10	82	2
	12	M	Initial	7.3	17.2	47	14,800	-	-	-	-	4	38	56	2
			1 month	8.8	16.8	50	13,500	2	-	-	-	-	39	66	?
			2 months	9.8	17.2	48	16,000	1	-	-	-	-	19	80	-
			3 months	8.0	15.6	46	12,650	-	-	-	-	-	15	83	2
	13	M	Initial	7.8	16.2	46	15,800	-	-	-	-	1	36	62	1
			1 month	7.3	16.8	49	14,600	9	-	-	-	-	24	66	1
			2 months	8.9	16.0	50	15,000	3	-	-	-	-	16	77	4
			3 months	7.3	17.2	48	13,050	5	-	-	-	-	40	52	3
	14	M	Initial	7.3	16.2	45	17,200	1	-	-	-	2	31	65	2
			1 month	6.7	14.4	47	18,000	2	-	-	-	-	10	88	-
			2 months	8.1	16.5	48	19,000	1	-	-	-	-	26	70	3
			3 months	8.3	15.2	47	18,500	3	-	-	-	1	21	74	1
	15	M	Initial	7.4	16.4	45	8,400	-	-	-	-	-	8	91	1
			1 month	8.11	14.8	49	9,400	2	-	-	-	-	10	83	-
			2 months	8.7	15.0	50	10,350	-	-	-	-	-	12	88	-
			3 months	9.2	14.8	50	16,500	-	-	-	-	1	14	85	-
	16	M	Initial	7.7	15.6	47	19,500	1	-	-	-	3	13	83	1
			1 month	7.7	15.0	47	17,300	11	-	-	-	2	15	72	-
			2 months	9.1	15.2	51	15,200	2	-	-	-	-	22	76	-
			3 months	8.6	15.0	49	11,100	2	-	-	-	1	41	53	3
	17	M	Initial	7.3	16.0	43	19,200	2	-	-	-	3	19	75	1
			1 month	8.7	17.4	51	17,100	1	-	-	-	-	14	85	-
			2 months	8.9	16.2	48	18,100	-	-	-	-	-	29	70	1
			3 months	8.8	15.4	45	20,800	1	-	-	-	-	23	71	5
	18	M	Initial	7.8	15.8	48	8,200	-	-	-	-	1	36	63	-
			1 month	8.1	16.2	50	14,000	1	-	-	-	-	36	62	1
			2 months	8.8	16.0	50	9,800	-	-	-	-	-	37	61	2
			3 months	7.9	15.8	49	15,000	4	-	-	-	-	41	48	7

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TABLE VIII(Cont)
BLOOD PICTURE - RATS

Differential Count

Grp and Dose level	Rat No.	Sex		RBC in millions	Hemoglobin (gms per 100 cc)	Hemato-crit vol %	WBC	Eosino philes	Baso phils	Myelo cytes	Juven-iles	Stab	Segmented	Lympho cytes
Grp II	19	M	Initial	6.6	14.2	46	11,200	1	-	-	-	7	25	66
			1 month	8.7	15.4	51	11,700	2	-	-	-	-	22	76
			2 months	9.6	15.8	50	9,600	3	-	-	-	-	21	75
			3 months	8.6	16.4	49	12,500	3	-	-	-	1	26	67
	20	M	Initial	7.5	14.8	48	19,200	1	-	-	-	4	34	61
			1 month	9.0	17.2	55	16,400	2	-	-	-	-	19	78
			2 months	8.8	17.5	52	10,500	2	-	-	-	-	34	64
			3 months	8.5	16.8	50	12,500	1	-	-	-	-	21	71

TABLE IX

BLOOD PICTURE – RATS

GROUP III – CONTROL

Grp and Dose Level	Rat No.	Sex		RBC in millions	Hemoglobin (gms per 100 cc)	Hemato-crit vol %	WBC	Eosino philes	Baso philes	Myelo cytes	Juven iles	Stab	Segmen ted	Lympho cytes	Mono cytes
Control	1	F	Initial	7.2	14.4	48	16,000	-	-	-	-				-
			1 month	8.2	16.4	50	16,200	6	-	-	-	4	19	76	-
			2 months	8.7	16.6	51	17,100	2	-	-	-	-	25	69	-
			3 months	7.5	17.4	47	13,950	2	-	-	-	-	6	90	2
	2	F	Initial	6.6	15.3	43	10,800	2	-	-	-	1	6	90	1
			1 month	7.2	16.0	48	15,500	4	-	-	-	4	27	66	1
			2 months	8.8	17.8	52	15,000	4	-	-	-	1	27	67	1
			3 months	9.2	15.9	51	14,700	1	-	-	-	-	29	67	-
	3	F	Initial	6.6	15.2	43	12,600	-	-	-	-	-	40	53	6
			1 month	7.8	16.8	48	12,900	4	-	-	-	-	15	84	1
			2 months	7.7	18.1	48	16,000	-	-	-	-	1	14	79	2
			3 months	9.0	16.7	53	10,000	5	-	-	-	-	6	94	-
	4	F	Initial	7.0	14.8	44	8,800	-	-	-	-	-	31	58	6
			1 month	8.6	16.2	51	7,550	6	-	-	-	1	19	80	1
			2 months	8.1	17.2	51	12,450	-	-	-	-	-	32	61	1
			3 months	8.2	16.2	51	14,600	2	-	-	-	-	21	78	2
	5	F	Initial	6.3	14.4	43	10,900	-	-	-	-	-	27	69	-
			1 month	6.9	16.7	47	20,050	7	-	-	-	7	41	52	-
			2 months	8.6	17.2	50	18,800	-	-	-	-	-	20	73	-
			3 months	7.5	17.5	47	18,700	10	-	-	-	1	29	71	-
	6	F	Initial	7.7	15.3	48	16,100	-	-	-	-	4	23	66	-
			1 month	6.7	17.8	50	12,250	13	-	-	-	-	37	59	13
			2 months	8.9	17.8	52	18,900	2	-	-	-	-	28	58	2
			3 months	7.5	18.1	49	17,500	-	-	-	-	-	11	86	-
	7	F	Initial	6.8	15.1	42	9,500	-	-	-	-	-	34	64	-
			1 month	9.0	17.2	51	13,300	3	-	-	-	-	34	66	3
			2 months	9.3	17.8	47	14,700	3	-	-	-	-	26	71	3
			3 months	8.4	15.0	48	14,500	4	-	-	-	-	17	80	1
	8	F	Initial	7.8	15.2	47	16,400	-	-	-	-	4	9	86	-
			1 month	8.9	16.8	50	18,100	1	-	-	-	-	32	64	1
			2 months	8.5	16.6	47	21,650	4	-	-	-	1	20	79	4
			3 months	8.1	16.3	47	20,900	-	-	-	-	-	37	54	7

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TABLE IX (Cont)

BLOOD PICTURE - RATS
GROUP III - CONTROL

Differential Count

Grp and Dose Level	Rat No.	Sex		RBC in millions	Hemoglobin (gms per 100 cc)	Hemato-crit vol %	WBC	Eosino-philes	Baso phils	Myelo cytes	Juven iles	Stab	Segmen ted	Lympho cytes	Mono cytes
Group III Control	9	F	Initial	7.8	16.3	48	10,100	-	-	-	-	1	39	59	-
			1 month	7.3	17.3	49	8,000	5	-	-	-	-	22	72	5
			2 months	7.7	16.8	46	15,900	1	-	-	-	-	31	68	1
			3 months	8.3	17.8	50	15,000	3	-	-	-	-	24	66	7
	10	F	Initial	7.5	16.2	48	13,600	-	-	-	-	3	33	64	-
			1 month	8.9	16.4	51	12,100	12	-	-	-	-	32	54	12
			2 months	8.9	18.0	51	19,750	6	-	-	-	-	22	71	6
			3 months	8.7	17.3	50	15,500	5	-	-	-	-	27	66	2
	11	M	Initial	7.9	15.9	48	12,300	-	-	-	-	4	44	52	-
			1 month	8.3	17.2	50	19,300	1	-	-	-	-	21	78	-
			2 months	9.1	16.8	49	19,800	2	-	-	-	-	55	43	-
			3 months	7.8	15.0	47	15,700	-	-	-	-	-	20	79	1
	12	M	Initial	6.8	14.2	45	13,000	-	-	-	-	2	30	68	-
			1 month	7.8	16.2	50	10,000	4	-	-	-	-	16	79	1
			2 months	8.1	17.6	51	15,900	1	-	-	-	-	25	73	1
			3 months	7.8	16.4	46	12,500	7	-	-	-	-	28	63	2
	13	M	Initial	6.3	14.6	44	19,000	2	-	-	-	1	35	62	-
			1 month	7.4	16.8	47	17,300	3	-	-	-	-	20	77	-
			2 months	8.4	15.0	50	17,400	6	-	-	-	-	22	72	-
			3 months	9.2	15.7	48	14,700	3	-	-	-	-	14	83	-
	14	B	Initial	7.4	15.2	47	13,600	-	-	-	-	1	39	60	-
			1 month	7.9	16.2	50	18,000	1	-	-	-	-	16	81	2
			2 months	9.0	16.8	51	10,300	1	-	-	-	-	19	80	-
			3 months	8.3	16.4	46	12,350	4	-	-	-	-	27	67	2
	15	M	Initial	7.5	14.8	46	14,800	3	-	-	-	3	23	71	-
			1 month	6.5	16.6	51	16,500	7	-	-	-	1	29	59	4
			2 months	8.2	16.4	54	17,900	1	-	-	-	-	27	72	-
			3 months	9.1	15.4	50	15,500	4	-	-	-	-	35	58	3
	16.	M	Initial	7.6	14.8	49	12,000	1	-	-	-	-	24	75	-
			1 month	8.4	16.0	53	11,050	2	-	-	-	-	18	79	1
			2 months	8.9	17.0	53	10,460	1	-	-	-	-	33	66	-
			3 months	8.4	16.8	52	15,800	1	-	-	-	-	19	79	1

TABLE IX(cont)
BLOOD PICTURE - RATS
GROUP III - CONTROL

Differential Count

Grp and Dose level	Rat No.	Sex		RBC in millions	Hemoglobin (gms per 100 cc)	Hematocrit vol %	WBC	Eosinophiles	Basophils	Myelocytes	Juveniles	Stab	Segmented	Lymphocytes	Monocytes
Group III Control	17	M	Initial	8.9	18.0	57	16,400	-	-	-	-	8	29	62	1
			1 month	7.9	15.8	49	17,300	3	-	-	-	-	22	71	4
			2 month	8.1	16.3	50	14,900	3	-	-	-	-	17	80	-
			3 months	8.7	16.0	49	15,150	1	-	-	-	-	11	88	-
	18	M	Initial	6.3	14.2	44	20,100	-	-	-	-	3	15	82	-
			1 month	8.4	18.0	54	15,000	-	-	-	-	1	20	78	1
			2 months	8.5	16.2	51	16,400	1	-	-	-	-	35	64	-
			3 months	7.3	16.7	48	19,000	2	-	-	-	-	38	58	2
	19	M	Initial	7.5	16.2	47	9,100	1	-	-	-	3	32	64	-
			1 month	6.7	17.2	47	12,500	3	-	-	-	2	25	69	1
			2 months	8.4	17.0	47	12,800	6	-	-	-	-	44	48	2
			3 months	8.3	16.0	49	19,700	4	-	-	-	-	39	53	4
	20	M	Initial	7.4	15.2	46	11,000	-	-	-	-	1	29	70	-
			1 month	9.3	17.0	59	13,000	1	-	-	-	-	12	87	-
			2 months	8.7	17.6	50	9,300	-	-	-	-	-	11	89	-
			3 months	8.9	15.7	50	15,000	3	-	-	-	-	23	73	1

TABLE X

RAT URINE

GROUP I - High Dose of Sample A(10mg/Kg)

Group	Rat No.	SUGAR Initial	1 mo.	2 mo.	3 mo.	ALBUMIN Initial	1 mo.	2 mo.	3 mo.	ACETONE Initial	1 mo.	2 mo.	3 mo.
Grp I A	1	N	N	N	N	N	N	N	N	N	N	N	N
	2	N	N	N	N	N	N	N	N	N	N	N	N
	3	N	N	N	N	N	N	N	N	N	N	N	N
	4	N	N	N	N	N	Tr	N	N	N	N	N	N
	5	N	N	N	N	N	N	Tr	N	N	N	N	N
	6	N	N	N	N	Tr	N	N	N	N	N	N	N
	7	N	N	N	N	N	N	Tr	N	N	Tr	N	N
	8	N	N	N	N	N	N	N	Tr	N	N	N	N
	9	N	N	N	N	N	N	Tr	N	N	N	N	N
	10	N	N	N	N	N	N	N	N	N	N	N	N
	11	N	N	N	N	N	Tr	Tr	N	N	Tr	N	N
	12	N	N	N	N	Tr	Tr	N	N	Tr	Tr	N	N
	13	N	N	N	N	N	N	Tr	N	Tr	N	N	N
	14	N	N	N	N	N	N	N	N	N	N	N	N
	15	N	N	N	N	N	N	Tr	N	N	N	N	N
	16	N	N	N	N	N	N	N	N	N	N	N	N
	17	N	N	N	N	N	N	N	N	N	N	N	N
	18	N	N	N	N	N	Tr	Tr	N	N	N	N	N
	19	N	N	N	N	N	Tr	N	N	N	N	N	N
	20	N	N	N	N	N	Tr	N	N	N	N	N	N

TABLE XI

RAT URINE

GROUP II – High Dose of Sample A (5 mg/Kg)

Group	Rat No.	SUGAR				ALBUMIN				ACETONE			
		Initial	1 mo.	2 mo.	3 mo.	Initial	1 mo.	2 mo.	3 mO.	Initial	1 mo.	2 mo.	3 mo.
Crp II	1	N	N	N	N	N	N	N	N	N	N	N	N
	2	N	N	N	N	N	N	N	N	N	N	N	N
B	3	N	N	N	N	N	Tr	N	N	N	N	N	N
	4	N	N	N	N	N	Tr	N	N	Tr	N	N	N
	5	N	N	N	N	N	N	N	N	N	N	N	N
	6	N	N	N	N	N	N	N	N	N	N	N	N
	7	N	N	N	N	N	N	N	N	N	N	N	N
	8	N	N	N	N	N	N	N	N	N	N	N	N
	9	N	N	N	N	N	N	N	N	N	N	N	N
	10	N	N	N	N	N	N	N	N	N	N	N	N
	11	N	N	N	N	N	N	N	N	N	N	N	N
	12	N	N	N	N	N	N	Tr	N	N	N	N	N
	13	N	N	N	N	Tr	Tr	N	N	N	N	N	N
	14	N	N	N	N	N	N	N	N	N	N	N	N
	15	N	N	N	N	N	N	N	N	N	N	N	N
	16	N	N	N	N	N	Tr	Tr	N	N	N	N	N
	17	N	N	N	N	N	N	N	N	N	N	N	N
	18	N	N	N	N	N	N	N	N	N	N	N	N
	19	N	N	N	N	N	N	N	N	N	N	N	N
	20	N	N	N	N	N	Tr	Tr	Tr	N	N	N	N

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TABLE XII
RAT URINE
GROUP III – CONTROL

Group	Rat No.	SUGAR Initial	1 mo.	2 mo.	3 mo.	ALBUMIN Initial	1 mo.	2 mo.	3 mo.	ACETONE Initial	1 mo.	2 mo.	3 mo.
C	1	N	N	N	N	N	N	Tr	N	N	N	N	N
Control	2	N	N	N	N	Tr	Tr	Tr	Tr	N	N	N	N
	3	N	N	N	N	N	N	Tr	N	N	N	N	N
	4	N	N	N	N	N	N	Tr	N	N	N	N	N
	5	N	N	N	N	N	N	N	N	N	N	N	N
	6	N	N	N	N	N	N	Tr	N	N	N	N	N
	7	N	N	N	N	N	N	N	N	N	N	N	N
	8	N	H	N	N	N	N	N	N	N	N	N	N
	9	N	N	N	N	N	N	Tr	Tr	N	N	N	N
	10	N	N	N	N	N	N	N	N	N	N	N	N
	11	N	N	N	N	Tr	Tr	N	Tr	Tr	N	N	N
	12	N	N	N	N	N	N	N	N	N	N	N	N
	13	N	N	N	N	N	N	N	N	N	N	N	N
	14	N	N	N	N	N	N	Tr	N	Tr	N	N	N
	15	N	N	N	N	N	N	N	N	N	N	N	N
	16	N	N	N	N	N	Tr	N	N	N	N	N	N
	17	N	N	N	N	N	N	Tr	Tr	N	N	N	N
	18	N	N	N	N	N	N	Tr	N	N	N	N	N
	19	N	N	N	N	N	N	N	N	N	N	N	N
	20	N	N	N	N	Tr	Tr	N	N	N	N	N	N

TABLE XIII

AVERAGE BLOOD PICTURE

Group			RBC in millions	Hemoglobin (gms per 100 cc)%	Hemato crit vol %	WBC	Eosino philes	Baso phils	Myelo cytes	Juven iles	Stab	Segmen ted	Lympho cytes	Mono cytes
Group I A	Female 1 - 10	Initial	7.84	16.19	46.9	14,670	0.7	-	-	-	1.3	27.8	65.2	0.7
		1 month	8.06	17.46	51.5	13,360	5.7	-	-	-	0.4	28.3	65.7	4.6
		2 months	8.13	17.10	49.9	14,675	2.6	-	-	Z	0.4	31.3	64.3	2.6
		3 months	8.41	17.51	50.2	14,580	2.9	-	-	Z		28.3	63.6	2.2
	Male 11 - 20	Initial	7.33	15.95	46.2	12,170	0.5	-	-	-	-	28.3	63.6	2.2
		1 month	7.86	16.68	50.7	16,375	2.5	-	-	-	0.8	35.3	59.7	2.8
		2 months	8.32	16.39	51.3	16,125	1.3	-	-	-	0.4	18.9	75.8	1.4
		3 months	7.72	15.69	49.7	15,560	2.2	-	-	-	0.2	20.4	76.2	1.9
	Average Male and Female 1 - 20	Initial	7.48	16.07	46.5	13,420	0.6	-	-	-	-	20.4	75.7	1.6
		1 month	7.96	17.07	51.1	14,867	4.10	-	-	-	1.05	31.55	62.95	1.75
		2 months	8.22	16.74	50.6	15,400	1.95	-	-	-	0.40	23.60	70.75	3.00
		3 months	8.06	16.60	49.9	15,070	2.55	-	-	-	0.30	25.85	70.25	2.25
Group II B	Female 1 - 10	Initial	6.79	16.07	42.3	15,095	0.1	-	-	-	-	24.35	69.96	1.90
		1 month	7.56	16.08	47.5	15,730	4.0	-	-	-	0.9	29.9	59.3	0.7
		2 months	8.40	15.70	49.6	15,885	1.8	-	-	-	0.2	25.5	68.3	1.3
		3 months	8.21	15.63	48.4	14,260	2.2	-	-	-	-	24.6	72.2	2.0
	Male 11 - 20	Initial	7.49	15.80	45.9	14,380	0.6	-	-	-	0.3	22.0	72.8	2.7
		1 month	8.15	16.02	50.1	14,500	3.2	-	-	-	2.7	28.2	67.6	0.8
		2 months	8.94	16.15	49.9	13,755	1.5	-	-	-	0.2	21.6	73.2	0.3
		3 months	8.10	15.72	48.2	14,900	1.9	-	-	-	-	24.1	73.3	1.1
	Average Male and Female 1 - 20	Initial	7.14	15.93	44.1	14,737	0.35	-	-	-	0.4	25.2	68.6	3.3
		1 month	7.85	16.05	48.8	15,115	3.60	-	-	-	1.80	29.05	63.45	0.75
		2 months	8.67	15.92	49.7	14,820	1.65	-	-	-	0.20	23.55	71.75	0.80
		3 months	8.15	15.67	48.3	14,580	2.05	-	-	-	0.15	24.35	72.75	1.50
Group III Control	Female 1 - 10	Initial	7.13	15.22	45.4	12,480	0.2	-	-	-	0.35	23.60	70.70	3.00
		1 month	7.95	16.76	49.5	13,595	6.1	-	-	-	2.8	29.6	67.0	0.3
		2 months	8.52	17.39	49.5	17,025	2.2	-	-	-	0.2	24.6	68.3	3.8
		3 months	8.24	16.82	49.3	15,535	3.2	-	-	-	0.1	20.6	76.4	1.9
	Male 11 - 20	Initial	7.36	15.31	47.3	14,130	0.7	-	-	-	0.2	25.8	67.2	3.2
		1 month	7.86	16.70	51.0	15,041	2.5	-	-	-	2.3	30.0	66.6	0.1
		2 months	8.64	16.67	50.6	14,510	1.9	-	-	-	0.4	19.9	75.8	1.4
		3 months	8.38	16.01	48.5	15,540	2.9	-	-	-	-	28.8	68.7	0.3
	Average Male and Female 1 - 20	Initial	7.24	15.26	46.3	13,305	0.45	-	-	-	-	25.4	70.1	1.6
		1 month	7.90	16.73	50.2	14,318	4.30	-	-	-	2.55	29.80	66.80	0.20
		2 months	8.58	17.03	50.0	15,767	2.05	-	-	-	0.30	22.25	72.05	2.60
		3 months	8.32	16.41	48.9	15,537	3.05	-	-	-	0.10	25.60	68.66	2.40

OBSERVATIONS ON THE INTERNAL CORROSION OF TIN-PLATE CANS BY ACID FOODSTUFFS

By H. CHEFTEL, J. MONVOISIN and MALWINA SWIRSKI

By calculating from Nernst's equation the potentials of tin and iron, it is shown that the anodic behaviour of tin vs. iron in the corrosion of tin plate by canned foods is what would be expected on theoretical grounds. With reference to the fact that the hydrogen evolved during the corrosion of tin plate is less than the amount corresponding to the metals dissolved, an electrochemical mechanism is suggested which may account in part for this deficit. Attention is also drawn to previously unsuspected accelerators of corrosion, e.g. caramelization products in fruits. It is interesting to note that while caramel from dextrose acts as an efficient accelerator, caramel from sucrose has no effect.

Potential of tin vs. iron

It has long been known, since the pioneering studies of Mantell & King,[1] Kohman & Sanborn,[2,3] Clark,[4] Lueck & Blair,[5] Culpepper & Moon,[6] and others, that in the corrosion of unlacquered tin-plate cans by acid products such as fruits, the tin is anodic towards the steel base.

Differences of opinion have, however, been expressed as to the explanation of this so-called 'inversion' of the respective potentials of tin and iron.

More recently, Hoar[7] has confirmed that tin assumes a negative potential towards iron in dilute acid solutions, even when the two metals are connected in a couple and also at pH lower than those given by Kohman & Sanborn.

The latter authors have rightly remarked[2] that ' the relative positions of the metals in the electrochemical series do not necessarily indicate their potentials in various media ', but no-one has apparently attempted, as far as we know, to calculate the potentials of tin and iron in weak acid media. When this is done, as shown below, it is apparent that there is no longer any need to speak of a reversal of the potentials; in weak acid solutions, tin simply assumes a more electronegative potential than iron, and no reversal of potentials appears to occur. Whatever reversal of potentials has been observed[7] has been from the oxides to the metals, but not from the normal potentials as defined in the electrochemical series.

Calculation of the potentials

The potential which a divalent metal takes in a solution of its own ions, is given by the formula :

$$E = E_0 + \frac{0 \cdot 06}{2} \log [M^{++}] \qquad \qquad (1)$$

where E_0 is the normal potential and $[M^{++}]$ the concentration of metal ions in the solution.

For tin, the above formula becomes :

$$E_{Sn} = E_{0_{Sn}} + 0 \cdot 03 \log [Sn^{++}]$$
$$= -0 \cdot 136 + 0 \cdot 03 \log [Sn^{++}] \ . \qquad \qquad (2)$$

The value of $\log [Sn^{++}]$ may be calculated, for pH between 2·0 and 11·0, from the solubility product of $Sn(OH)_2$:

$$[Sn^{++}] [OH^-]^2 = 5 \times 10^{-26}$$

whence
$$\log [Sn^{++}] = \log 5 - 26 - 2 \log [OH^-]$$
or, as
$$\log [OH^-] = pH - 14,$$
$$\therefore \log [Sn^{++}] = \log 5 - 26 - 2 (pH - 14)$$
$$= 0 \cdot 7 - 26 + 28 - 2 \ pH$$
$$= 2 \cdot 7 - 2 \ pH$$

Substituting this value in equation (1) we finally get :

$$E_{Sn} = -\ 0.136 + 0.03\ (2.7 - 2\ pH)$$
$$= -\ 0.056 - 0.06\ pH$$

In the case of iron, we have similarly :

$$E_{Fe} = E_{0_{Fe}} + 0.03\ \log\ [Fe^{++}]$$
$$= -\ 0.44 + 0.03\ \log\ [Fe^{++}]$$

and (for pH between 5.5 and 11.0 only)

$$[Fe^{++}][OH^-]^2 = 1.65 \times 10^{-15},$$

whence

$$E_{Fe} = -\ 0.04 - 0.06\ pH$$

It is thus apparent that, at least for pH between 5.5 and 11.0, tin is always anodic with respect to iron. This behaviour is in no way extraordinary and results from the low solubility of Sn(OH)$_2$.

The fact that the above calculation is not valid at pH below 5.5 does not present a serious limitation ; in fact it is well established from experimental data that tin is still anodic to iron for pH much lower than 5.5. However, in order to calculate the potential of iron in the range of pH between 2 and 5.5, one would have to introduce the solubility products of ferrous salts—some of which form complexes—which form in solution, as well as the concentration of acid corrected for its dissociation coefficient.

It is not deemed necessary to embark on such a problematical calculation ; in fact, phenomena occurring in the corrosion of tin plate by food products are so intricate that no set of calculations, however elaborate, can be expected to give the full picture.

Disappearance of hydrogen

Another experimental observation which has given rise to much discussion is the fact that the amount of hydrogen accumulating in a tin-plate can is usually lower than the amount which would correspond to the tin and iron which have passed into the contents of the can.

It is known that atmospheric oxygen entrapped in the can, or hydrogen acceptors, may combine with hydrogen, the best example being probably provided by methylamine oxide in fish,[*] which is quantitatively transformed into methylamine, no hydrogen being evolved at all.

Not in all cases, however, do hydrogen acceptors account for the non-appearance or deficit of hydrogen. Diffusion of hydrogen ions through steel has been suggested sometimes as the explanation of this fact, but there is no definite proof in the case of tin-plate cans.

It is postulated, however, that there is another mechanism which may account, at least in part, for the disappearance of hydrogen.

In the unlacquered tin-plate can, tin is dissolved as the anode of the Fe/Sn couple and of other couples which may occur, such as tin impurities in the tin.

At the pH obtaining in canned foods, tin ions are present as positively-charged sols resulting from the peptization of SnO$_2$ in acid media, and it is actually in the form of the voluminous SnO$_2$,Sn^{++} complex[9] that they are attracted and deposited on the cathodic areas. The well-known inhibitory action of stannous ions in the corrosion of tin plate may be attributed, in our opinion, to the cathodic polarization thus produced. From this moment onwards, and until new and large areas of steel are exposed as a result of the stripping of the tin coating, the corrosion proceeds at a much lower speed which is mainly dependent upon the passage of current through the deposited layer of Sn(OH)$_2$.

While the dissolution of tin brings cathodic protection to the steel base, the steel may nevertheless be corroded at exposed areas (pores or fractures of the tin coating) through the action of other couples, such as, for example, FeS Fe. Hoare & Havenhand[10] have shown that sulphides stimulate the anodic reaction of steel areas. Chyzewski & Skapski,[11] in a study of the FeS Fe couple in weak-acid media (pH 3.6–4.6), with or without the presence of air, have found the following values :

$E_{FeS/Fe}$ in nitrogen	÷ 0.21 volt
„ in air	− 0.13 volt

The iron behaves as anode in the first case and as cathode in the second one.

Couples of this kind have a higher e.m.f. than the Fe Sn couple, and their potential may further be increased by the stresses developed in the metal as a result of deformation. As a consequence, while the general pattern of corrosion in the can is the one resulting from the Fe/Sn couple in which tin is anodic, localized corrosion may well proceed along other paths bringing into play greater e.m.f. than the ones which are to be attributed to the Fe/Sn couple alone.

Such processes bring about, of course, the dissolution of metal while they produce nascent hydrogen. Now, as it is known that stannous oxide can be reduced by nascent hydrogen,[12] it appears quite possible that the hydrogen thus made available acts upon the $Sn(OH)_2$ deposited on the steel; the resulting tin, in a finely-divided or spongy state, becomes detached from the steel and cannot function as anode in the Fe Sn couple. Meanwhile, part of the available hydrogen is used up. The net result is a transfer, in two steps, of *metallic* tin from the tin coating to the product; the hydrogen evolved during the first step, $Sn \rightarrow Sn^{--} \rightarrow Sn(OH)_2$, is balanced by the hydrogen consumed during the second step, $Sn(OH)_2 \rightarrow Sn$, while the amount of tin in the can contents is increased.

The above explanation, which is advanced in order to account for the deficit of hydrogen, is derived from known facts, and appears at least as plausible as the one advocating the diffusion of hydrogen ions through the tin plate. We are convinced that the corrosion of tin-plate cans containing acid foods cannot be accounted for by simple reference to the Fe Sn couple; the steel base is a very complex substance, and a number of other couples are no doubt involved.

Caramelization as a factor in corrosion

The occasional observation of instances of abnormally rapid corrosion in jams and fruits in syrup, whose flavour pointed to a certain degree of caramelization, has led to an investigation of the possible rôle of caramel as a corrosion accelerator.

One experiment consisted in comparing the shelf-life at 37° of plain tin-plate cans which had been filled, either with a normal product or with the same product slightly caramelized by local overheating. The results are shown in Table I.

Table I

Comparison of corrosion rate in normal and caramelized products

Time of storage at 37°, months	Percentage of 'springers'					
	Cherries in syrup		Mirabelles* in syrup		Apricot jam	
	Normal	Caramelized	Normal	Caramelized	Normal	Caramelized
4	o	o	o	o	o	
5	o	o	o	o	o	9
6	o	o	o	o	o	18
7	5	45	o	100	o	55
8	45	100	o		o	80
9	45		o		o	100
10	45		o		o	
15	100		11		o	
			100		o	

* Mirabelles are small, round, yellow plums grown in eastern France.

Although the above experiment was carried out on only 20–25 cans for each series, the results are so striking, especially with mirabelles in syrup and apricot jam, that the indication they give may be held as valid.

Further experiments were made by carrying out hydrogen evolution tests. Strips of tin plate, taken from the central part of the same sheet and 10 × 1 cm. in size, were immersed in 50 ml. of de-aerated citric acid–sodium citrate solution, pH 3·6, with 30% added sucrose or dextrose, as such or caramelized. Six parallel tests were made at 37° in each case. Table II shows the results obtained.

Similar experiments with strained prunes (total solids 16%, pH 4·4) also demonstrated this sometimes dramatic influence of caramelization on corrosion velocity.

The fact that caramel prepared from sucrose had no effect on corrosion has been repeatedly confirmed, using both the citrate buffer and 0·1N-hydrochloric acid for immersion of the tin-plate.

Table II

Influence of caramel on the rate of hydrogen evolution

Sucrose	Sucrose + caramel from sucrose	Dextrose	Dextrose + caramel from dextrose, time for appearance of 5 ml. of H_2 (days)
N	N	N	10
N	N	N	10
N	N	N	16
N	N	N	15
N	N	N	15
N	N	N	15

N = No hydrogen in 8 weeks

Further experiments are in progress using other mono- and di-saccharides, in order to establish if the aldehydic group, so important in the Maillard and similar reactions, is essential for the accelerating effect of caramel on the corrosion of tin plate.

It may be worth while noting that while caramel from sucrose does not exhibit any action on the corrosion of tin plate, it exerts a powerful inhibition on the corrosion of iron in o·IN-hydrochloric acid.

Perhaps the main interest of these preliminary results is that they point to one more factor in the intricate problem of the corrosion of the tin-plate container by food products. Accidental formation of small amounts of caramel may occur quite easily during the treatment and processing of most products containing sugar, and this fact should make one extremely cautious in interpreting occasional observations from commercial practice, even where nothing has apparently been changed in the practice of the canning factory.

Conclusions

1. It is shown that the anodic behaviour of tin *vs.* iron in the corrosion of tin plate by acid foodstuffs, which was demonstrated experimentally many years ago, may be predicted theoretically by calculations from Nernst's equation.

2. Previous theories concerning the discrepancy between the amount of hydrogen evolved and that corresponding to the metal dissolved are discussed and an electrochemical mechanism is suggested to account partly for this fact.

3. A slight caramelization acts as a powerful agent in the acceleration of tin-plate corrosion by certain fruit products. Caramel from sucrose exerts no accelerating effect, whereas caramel from dextrose does. Possibly this property is associated with the presence of reducing groups in the sugar molecule.

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Caramel

ORIGIN OF THE CARBON DIOXIDE PRODUCED IN THE BROWNING REACTION OF EVAPORATED MILK [1]

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SUMMARY

To determine the relative importance of lactose caramelization in the browning of evaporated milk, uniformly labeled lactose was added to milk samples before sterilization. The CO_2 produced in the browning reaction was recovered as $BaCO_3$ and its specific activity was determined. It was found that about 4% of the total CO_2 produced by sterilization of milk at $242°$ F. for 15 min. can be traced to caramelization of lactose.

The browning of heated milk largely has been explained on the basis of the Maillard reaction (sugar-amino interaction) (1, 8, 9, 11). The contention of Kass and Palmer (1), that browning of heated milk is caused entirely by lactose caramelization, has lost considerable ground, but there are investigators (15, 17) who admit the possibility that both phenomena are involved.

Since both the Maillard reaction, through the Strecker degradation of the amino acids (2, 8, 12), and the non-amino heat degradation of lactose (2, 15), are known to generate carbon dioxide proportionately to the brown discoloration, it follows that the relative extent of the two reactions is clearly indicated by determining the amounts of gas produced by each.

EXPERIMENTAL PROCEDURE

The evaporated milk used in these experiments was obtained from a commercial condensery. The samples were removed from the conveyor before sterilization, immediately placed in ice-water, and the experiments completed on the same day.

Uniformly labeled C^{14} lactose was dissolved in water at $40°$ C. and 4 ml. of solution containing exactly 800 mg. of the radioactive compound were added to each 14 ½-oz. can of milk. An equal volume of milk had been previously removed so that the volume of headspace would not be altered. The cans were soldered, agitated to ensure complete mixing of the added material, and sterilized in a Fort Wayne pilot batch sterilizer with the reel continuously operating in the following manner: (a) Temperature raised to $220°$ F. as fast as possible (6 min.); (b) temperature raised from 220 to $242°$ F. at the rate of $3°$ F. per minute; (c) temperature maintained 15 min. or longer at $242 \pm 1°$ F., then cooled 15 min. in tap water in the sterilizer.

Recovery of CO_2. A special apparatus (Figure 1) was built, consisting of a steel clamp, a glass tube, and a hollow needle type stainless steel plunger. A
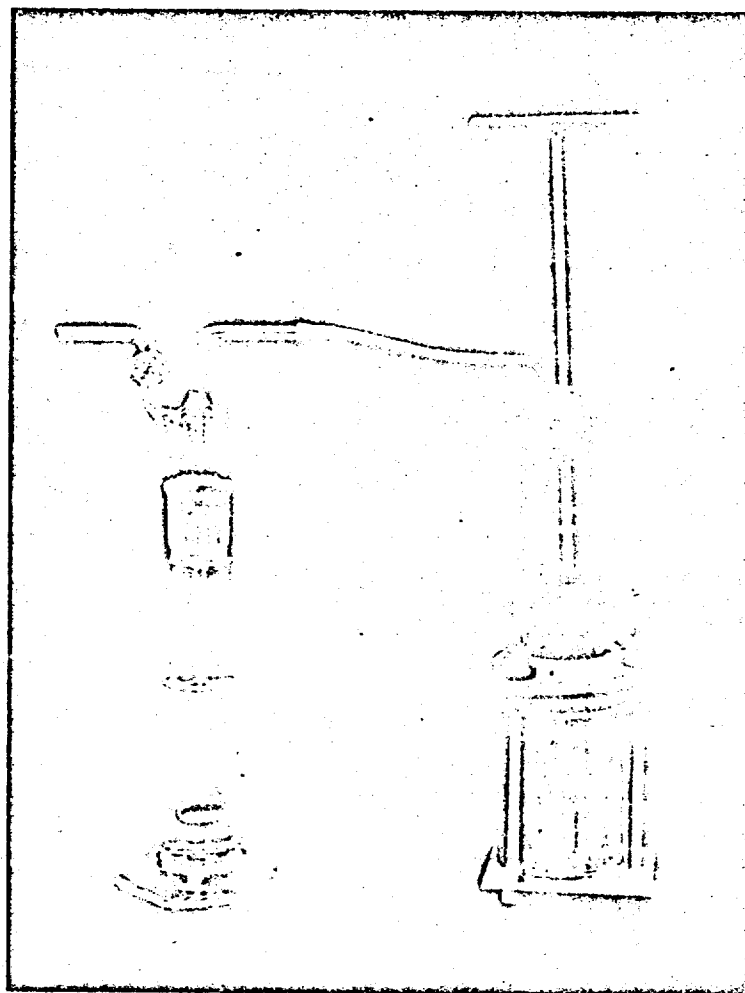
Fig. 1. Apparatus used for the recovery of CO_2.

If the can was put in the clamp, and the glass tube was placed on the can and tightened by means of screws. A resilient rubber gasket was inserted between the can and the glass tube, so that tightening the screws produced a gas-proof seal. The upper end of the glass tube was closed with a rubber stopper through which the hollow plunger passed, extending well into the tube with the tip touching the lid of the can. The upper end of the plunger was connected to a nitrogen cylinder. On gently hammering at the top with a wooden mallet, the sharp end of the plunger would perforate the lid of the can and could then be pushed to the bottom. Opening the valve of the nitrogen cylinder would force the milk well into the glass tube, with vigorous bubbling. A small amount of a silicon defoamer (Dow Corning Antifoam A; Dow Corning Corporation, Midland, Michigan) applied to the walls of the glass tube prevented excessive

foaming. The gases thus liberated were led into a gas scrubbing tower. This tower contained 100 ml. of 2 M NaOH, made at the time of each trial from a saturated stock solution and CO_2-free distilled water. Repeated runs showed that 60 min. of sweeping with N_2, keeping the can in 60° C. water bath, was necessary to recover practically all the CO_2 present. The contents of the scrubbing tower were then transferred to an Erlenmeyer flask, and 10 ml. of a precipitating mixture (0.25 M $BaCl_2$ and 5 M NH_4Cl) added. The material was left for about 15 min. in the 60° C. bath to accelerate the precipitation of $BaCO_3$. The precipitate was then filtered in a standard-size Tracerlab stainless-steel filter, and the planchets thus obtained were washed with 95% ethanol and dried to constant weight.

Radioactive assay. The specific activity of the planchets was measured with a flow-gas nuclear scaler. Because of the relatively low level of activity employed, each sample was counted for the period necessary to reduce the error to 2%.

Reflectance measurements. The reflectance of the milk was measured with a Beckman DU spectrophotometer equipped with reflectance attachment; powdered magnesium oxide was used as the standard white. Nelson (7) recommended that reflectance readings be made at wavelength 520 mμ, but better results were obtained at 400 mμ (Figure 2) because the greatest reflectance drop occurs at this wave length (Figure 3).

RESULTS AND DISCUSSION

The relationship between heat treatment and total amount of carbon dioxide produced (shown in Figure 4) exhibits a sigmoid curve quite similar to the one obtained by Tarassuk and Jack (16) for the production of the same gas
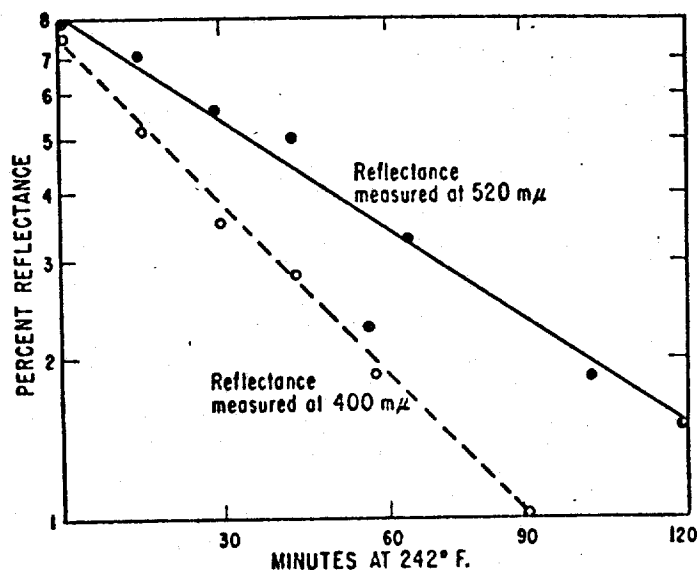
FIG. 2. Relationship between time of heating and reflectance.
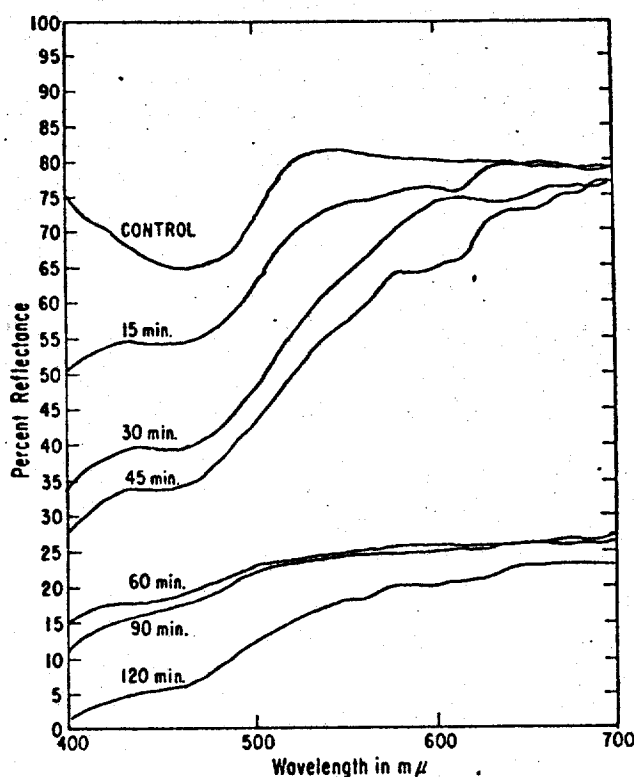
FIG. 3. Effect of duration of heating at 242° F. on the reflectance spectrum of evaporated milk.

during storage of air-packed whole milk powder of high moisture content. The value for amount of gas produced by heat treatment of 242° F. for 15 min. (66 mg. of BaCO₃) is in remarkable agreement with the value obtained by a direct absorptiometric technique (15). The correlation between carbon dioxide production and loss of reflectance is depicted (Figure 5). Summer milk of high heat stability was selected for this study, and it proved to be even more stable than was expected.

Results of the trials with radioactive lactose are given (Table 1).

TABLE 1

Effect of heat treatment on production of radioactive CO_2

Trial No.	Heat treatment (min. at 242° F.)	Specific activity (μc/mole of C)	CO₂ derived from lactose (%)
1	15	0.15	4.1
2	30	0.21	5.7
3	45	0.27	7.2

A 14 1/2-oz. can of evaporated milk contains about 40 g. of lactose (3). Therefore, addition of 800 mg. of radioactive lactose represents a 1:51 dilution of the labeled compound. The specific activity of the intact radioactive material
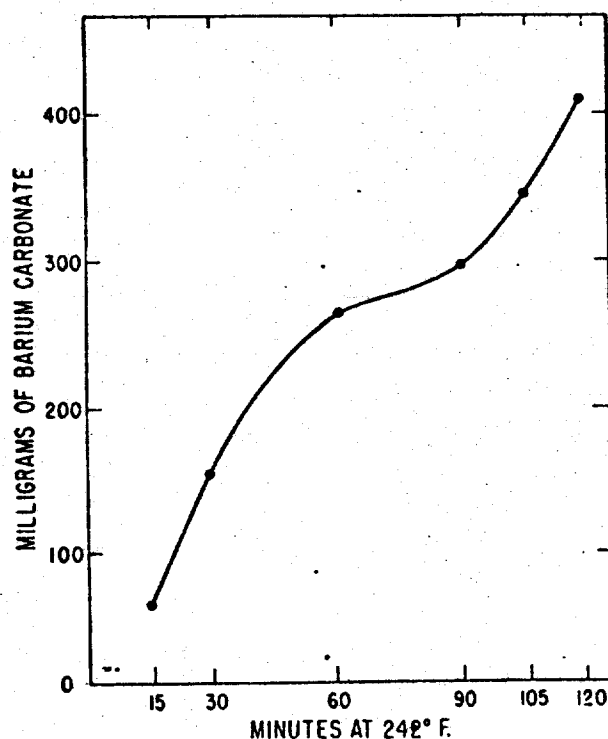
Fig. 4. Effect of heat treatment on total CO_2 production.

was 191.6 μc/mole of C, which means that, on the basis of dilution, the maximum possible activity of any planchet would be 191.6/51, or 3.7 μc/mole of C. and this reading would be obtained only if all of the CO_2 produced were coming from lactose breakdown.

The data show that caramelization is of minor importance in the browning of evaporated milk. The predominance of inert CO_2 points toward other pathways, the most important of which is undoubtedly the Maillard reaction. The contribution of this reaction could be quantitatively established by performing a set of identical trials, using carboxyl-labeled casein.

The data (Table 1) also suggest that the activation energy of caramelization is higher than that of the remaining pathways of CO_2 production, since the percentage of the gas traceable to lactose increased with increasing heat treatment of the milk.

The trial with radioactive lactose was repeated with half as much labeled compound of the same activity and the readings obtained were nearly one-half of those reported in Table 1.

It is well known that, in the case of dairy products, oxygen uptake parallels the production of carbon dioxide (11, 15, 16), but it has been amply demonstrated that browning proceeds independently of the oxygen present (5, 6, 13, 15). Browning by caramelization of lactose does require oxygen (15). However, it
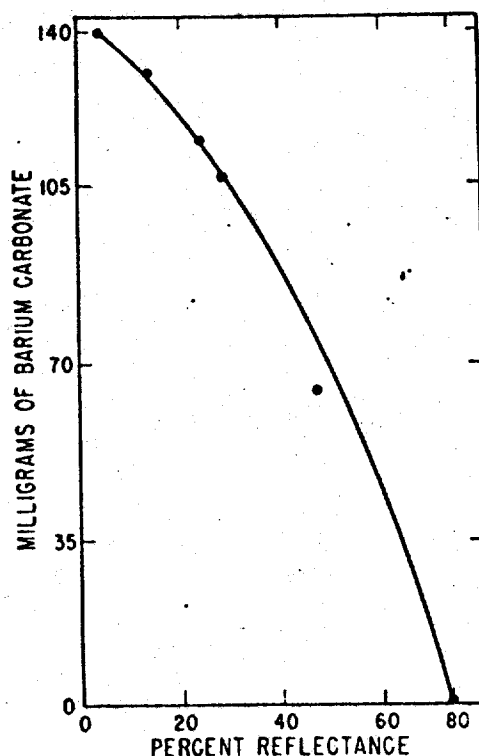
FIG. 5. Relationship between CO_2 evolution and reflectance loss.

is evident from the present data that only a small fraction of the oxygen used would be accounted for by caramelization. The loss of oxygen, in addition to that due to caramelization, could perhaps be explained in terms of the oxidation of sulfhydryl groups which have been shown to disappear on browning (10), and to oxidative changes of ascorbic acid.

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CANDY. See Confectionery.

CANNED GOODS. See Food.

CAPRIC ACID, $CH_3(CH_2)_8COOH$. See Fatty acids.

CAPROIC ACID, $CH_3(CH_2)_4COOH$. See Fatty acids.

ε-CAPROLACTAM (2-oxohexamethylenimine), $CH_2(CH_2)_4CONH$. See Cyclohexanol and cyclohexanone; Polyamides.

CAPRYLIC ACID, $CH_2(CH_2)_6COOH$. See Fatty acids.

CARAMEL COLOR

Caramel color or burnt-sugar coloring is the general trade name given to the heavy-bodied almost black syrup containing color components that impart the amber shade so extensively found in carbonated beverages, pharmaceutical and flavoring extracts, candies, soups, bakery products, and numerous other foods. This amber shade is produced whenever nitrogenous materials and sugars are cooked or processed together. For this reason it is one of the oldest food colors. This amber shade has become so fixed in our acceptance or evaluation of certain foods that when it is in low intensity it often causes the food or beverage to be rated inferior or lacking in strength. The housewife has always produced what she terms caramel for her own culinary needs by heating sucrose in a pan over an open flame. The sugar melts, water of constitution is lost, and the resulting hard material is used as a flavoring ingredient rather than a color, since its coloring power is small. Commercial caramel color is a vastly different material. Only a few drops are required to color six ounces of a carbonated beverage, and at this dilution there is no detectable contribution to flavor. Commercial caramel coloring can be produced by heating any saccharine material with a small amount of ammonia or ammonium salts. However, in practice, in order to produce a uniform color with definite analytical standards, the No. 70 and 80 corn sugars are generally employed (see Dextrose and starch syrups).

Caramel color is sold by the gallon and standardized in terms of tinctorial power (color intensity), Baumé, and other analytical standards generally associated with a specific type of coloring. Only a part of the syrup solids is the desired coloring; the balance is unreacted sugars. It is impossible to use all of the sugar for color. To do

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so would require excessive quantities of ammonia, resulting in a product with a very high tinctorial power but with attendant undesirable features, such as a tendency to resinify quickly on storage. Thus the manufacture of caramel color becomes a compromise between tinctorial power and caramel quality. Since the requirements for caramel quality vary widely in the many uses of caramel, it is often possible to "burn" a color to a higher tinctorial power (but other inferior qualities) which will serve satisfactorily in a given application but which would be entirely unsuited to a different application. These circumstances have led to considerable confusion, claim, and counterclaim, in the sale of caramel color. Since the amount of caramel color used in most applications is only a small amount of the total cost, it seems rather hazardous to engage in such practices.

It is difficult to ascertain the exact amount of caramel color produced annually in the United States. Some plants produce color for their own needs and offer the product for sale; others produce caramel color for sale only. However, it has been estimated that the production exceeds 100 million pounds.

In classification of caramel colors according to sugars used in the manufacture, there are three basic groups of coloring: cane-sugar caramel coloring, malt-caramel coloring, and corn-sugar or dextrose caramel coloring. The last is the most important product.

Cane-Sugar Caramel. This caramel is not manufactured in the United States, and is manufactured only to a limited extent in other countries. One foreign company manufactures this type of color for bonded distilled liquors. The product has a very low tinctorial power but will withstand concentrations of alcohol as high as 190 proof without clouding or precipitating. This caramel, however, when dissolved in water, will floc or precipitate with a trace of acid. The use of invert syrup, employing the usual catalysts, does not produce a satisfactory color. This failure is attributed to the instability of the levulose under the acidic conditions of the high temperature attained in the "burning" process.

Malt Caramel. This caramel is prepared from malt syrup or combinations of malt syrup and corn-sugar syrup. Since malt syrup contains considerable nitrogenous material, added ammonium salts are usually not necessary. The resulting caramel has a malty odor (bouquet) and flavor depending upon the type of malt syrup used and the manufacturing or "burning" procedure. Malt caramels are used in brewing to add color and flavor to beer. Since the current fashion is for very light amber beers, the production of this type of color is only a small fraction of what was formerly produced.

Corn-Sugar or Dextrose Caramel. This is the important coloring of commerce in the U.S. and in other countries. The requirements for a good beverage caramel color are so exacting that a good starting sugar base is essential. For this purpose the No. 70 and 80 corn sugars are ideal. The former is more generally used since the solid chipped sugar is re-formed more readily by heat into a syrup, thus enabling the "burning" cycle to be completed in a shorter time. In recent years a large amount of "first greens" (the residual syrup from the first centrifugation of crystalline dextrose) has been used (see Sugar manufacture). The ash content of this material is much higher than the ash of "70" sugar but its lower cost has prompted its use for certain types of caramel coloring. More recently, polymerized dextrose syrups have been offered. These syrups are covered by patents (1,5). These polymerized corn-sugar syrups are produced by the acid-heat treatment of corn syrups and corn-sugar syrups (6). The syrups are noncrystallizing and can be shipped in the usual tank cars, thereby eliminat-

ing the costs of crystallizing, chipping, and bagging the sugar, and the subsequent "melting" costs by the caramel manufacturer. Polymerization of dextrose occurs in the usual "burning" process and is necessary for the formation of a quality caramel. Thus the use of this type of syrup necessitates carrying out this part of the caramel reaction outside of the kettle, thus decreasing the time required for a batch of caramel.

Physical and Chemical Properties

Tinctorial Power. The tinctorial power, or the amount of coloring per pound of syrup, is important to the seller and the user of the color. Therefore the method for measurement of the coloring power is of the utmost importance. A 1% solution of a beverage caramel appears to be red by transmitted light rather than the desired amber shade obtained by further dilution. For this reason it is best that the measurement of the coloring power be carried out at about the concentration at which it is used. This condition is attained when one gram of caramel color is diluted to one liter. This solution is amber and can be effectively measured through a one-inch cell with Lovibond No. 52 caramel glass slides (see also Color measurement). This method for color measurement was established many years ago by Lovibond and it is still judged to be the best system. The tinctorial power has often been the pawn in the sale and purchase of caramel color—a little more color for the same price per gallon. This increase in coloring power is often attained by a deterioration in one or more of the other qualities that make a good caramel color. For this reason any numerical statement of the tinctorial power of caramel color will not meet with the approval of everyone. A satisfactory tinctorial power for beverage and brewers'.colors is 22, and for bakers' and confectioners' caramel, 30 is satisfactory.

Specific Gravity. The sp gr of a caramel color will vary from 30 to 40° Bé, although most of the products will fall within the range 36–40° Bé and will average about 38 Bé. This wide variation in sp gr results from the efforts of the manufacturer to adjust the three variables of solids, tinctorial power, and viscosity. The solids must be high enough to prevent fermentation, although the pH of the syrup is also an important factor in whether the syrup does or does not ferment. The tinctorial power is extremely important to the user; he desires a product that is essentially the same from lot to lot. If a manufacturer cannot produce a color to a predetermined tinctorial power, he usually chooses to keep the coloring power constant, achieving this by altering the amount of water added to the hot caramel as it comes from the kettle. The viscosity of a caramel color is greater than the viscosity of the starting sugar syrup (both measurements at the same Baumé). This thickening of the syrup through the formation of the color is normal to the "burning" process; the extent of the thickening is dependent upon the type of caramel being produced, the catalyst employed, and the kettle-procedure followed. For this reason the viscosity of caramel color varies between manufacturers and this property becomes an important consideration in storage, transfer, and measurement, particularly in cold weather. If the manufacturer cannot achieve a workable viscosity at the usual specific gravities, he must resort to the addition of water to obtain a satisfactory product. The viscosity of all caramel colors increases on storage or ageing. However, there is a wide difference in this property between producers. Some colors increase in viscosity very slowly; others rapidly, and in some instances the syrup has been known to resinify in the barrel.

Dry Substance. The dry substance of a caramel color cannot be determined by the usual oven procedures since the heat will continue the color reaction with a further

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release of water of constitution. The best analytical method for the determination of moisture is benzene distillation. Using this method, the moisture of a 38 Bé caramel is about 30%.

Odor. The odor of caramels used in carbonated beverages is slightly pungent or acrid. The odor of the bakers' and confectioners' and brewers' colors is usually less because the pH of these products is greater than for the beverage caramels.

pH. The pH of caramel will vary from 2.7 to 3.3 for acid-fast beverage color, from 3.2 to 3.6 for foaming-beverage caramel, and from 4.0 to 4.5 for bakers' and brewers' colors.

Special precautions are necessary for the shipment and storage of beverage caramel color because this product is distinctly acidic. All steel pails, barrels, and drums must be protected by a suitable liner. Large storage tanks should be fabricated from stainless-clad steel. Attempts to line a steel tank after fabrication have not been too successful.

Ammonia Content. The dry-basis ammonia content varies considerably, not only with respect to type of color, but also with the manufacturer. Carbonated-beverage caramels have been analyzed with an ammonia content as low as 0.2% and as high as 1.2%, for a highly acid-resistant beverage caramel. True beer colors and bakers' caramel will contain as much as 2.0%.

Ash Content. The ash content of caramel color varies widely, depending to a large extent on the ash content of the starting sugar. The ash content of No. 70 is about 0.6%, dry basis. The condensation of the dextrose during the "burning" reaction will increase the ash in proportion to the water lost. The salts used as catalysts may increase the ash content. The ash content of first greens, largely sodium chloride, varies between 2.0 and 2.5%, and this ash remains in the finished caramel color when it is made from this starting sugar.

Acidfastness. This is the term applied to colors used for carbonated beverages, particularly colas and ginger ales. The term is often overworked, for beer colors and bakers' caramel are, as a rule, more acid-fast than the carbonated-beverage colors. Acidfastness is pertinent only when considered with the equally important property of tannin resistance, for a good beverage caramel must have both. Again, the degree of acidfastness is relative, for it is obvious that a color used for root beer, which is only mildly acidic, need not be as great as one used for cola beverages. Further the acid-fastness of a caramel used for fountain colas need not be as great as a color used for bottled goods which may have a shelf life of a month or more.

Foaming Properties. In using caramel colors in carbonated beverages, the foaming properties of the colors must be carefully evaluated. Bottlers of colas prefer a color that imparts a small amount of foam that breaks readily when the liquid is poured from the bottle into a glass. The makers of cream soda and the mug-type root beer desire more foam, in fact with the latter drink they prefer a fine lacey head that will persist for some time. Foaming properties can be built into a caramel color, but at the expense of acidfastness.

Stability to Alcohol. Stability to alcohol is desired for colors used in imitation vanilla extract and in many pharmaceutical syrups and extracts, and for colors added to distilled liquors. Small-scale testing, using the actual amounts of color required, necessitates extended periods of time to be sure that the color will not floc out. An accelerated test has been developed that replaces the time-consuming testing under conditions of use. This test is based on adding 1 g of color to 100 ml of alcoholic

solutions. A good caramel color will remain clear indefinitely in 50% alcohol; some colors will remain clear in 60% solutions.

Neutral Tannin Test. This test has proved to be invaluable in determining whether a color will be precipitated by the various organic extractives occurring in flavoring and pharmaceutical extracts. A caramel must have a good tannin resistance to be rated as satisfactory for extract use.

Iron Content. The iron content of beverage and beer colors should not exceed 10 ppm. The iron content of No. 70 sugar is about 5 ppm and if stainless steel equipment is employed, there should be no problem in keeping the iron content below the limit given.

Chemistry. Very little has been published on the chemistry of caramel color since an early article by Salamon and Goldie (10). Two explanations may be given for this dearth of published information: (a) the producers of caramel color have always operated their art as a secret process, with no desire for the release of any information, and (b) others who have been interested in obtaining basic information have dropped their investigations when the complexity of the problem became apparent. Caramel color is produced by two reactions, proceeding simultaneously: polymerization of the dextrose under conditions of acid and heat, resulting in the release of water, and the reaction of ammonia with the aldehyde group to form the aldehyde ammonia, followed by a rearrangement and condensation, again with the release of water. The consideration of these two reactions will explain, at least in part, the difficulties encountered in the "burning" process. The reactions also show that a considerable loss of sugar solids occur.

Recently the polymerization of dextrose under an acid and heat treatment has been studied extensively (6). With high concentrations of dextrose the reducing power is decreased and the specific rotation of the sugars and the viscosity of the syrup are increased. This condition occurs in the manufacture of caramel color from No. 70 corn sugar. Starting with a DE (dextrose equivalent, ie, reducing sugars calculated as dextrose and expressed on a dry substance basis) of about 84, the DE may decrease to as low as 25 in extreme conditions. A good general rule is that a satisfactory beverage caramel will not be obtained unless the DE is below 45.

Patents have been issued (4,7,9) for the manufacture of caramel color by a two-step process. The first step consists of the treatment of the sugar syrup under conditions of acid, heat, and time to reduce the DE to about 45. This acidulated syrup is then treated with ammonia and other ammonia salts and further processed to produce the caramel color reaction. The inventors claim these methods of producing caramel color result in colors which are more uniform in tinctorial power and the other qualities which make for a better color.

Attempts have been made to determine the various constituents in caramel color by means of paper chromotography. However the plethora of constituents revealed in the preliminary test indicates the complexity of the product and usually ends the desire for more detailed study. The dialysis of caramel color will separate a color body of very high tinctorial power; this contains essentially all of the ammonia used in the preparation of the color.

Manufacture of Dextrose Caramel Color

Caramel color is produced in two types of kettles—open and closed. The former is the original type. It has passed through a variety of methods for the application of

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heat includinig coke-fired, gas-fired, Dowtherm, and high-pressure steam (ca 150 lb pressure). One burner still employs the coke-fired kettle, and it is a rather remarkable experience to watch the skill of the operator in starting and banking the fire so that excessive charring is avoided, for the kettle has no stirring mechanism. The direct-fired kettle always produces char and the process gave the product its original name, burnt-sugar coloring.

Open-Kettle Process. The newer open kettles are from 6 to 8 ft in diameter, usually stainless clad and jacketed. The stirring mechanism is dual countercurrent action, with scraper blades attached to the outer arms. The kettle is closed by a cover, through which a large stack, usually 18–24 in. in diameter, leads to the roof. A thermocouple, usually leading to a recorder, registers the temperature of the heated syrup. The recorder chart usually contains space for two or more cycles. Thus the operator can alter the immediate curve, if necessary, according to analytical information obtained on a previous batch. The elapsed time is about 4 hr.

The kettle is loaded with 4000–6000 lb of sugar syrup and steam is applied to the jacket. The syrup boils and is concentrated. At a definite time or concentration, a solution of catalyst is fed through the bottom of the kettle into the heated syrup. The temperature of the syrup increases as the solids concentration increases. At about 6% water the caramel-color reaction starts. Water is released from the polymerization of the dextrose and from the color reaction. This water plus the small amount from the solution of the catalyst keeps the concentration of the solids relatively constant, resulting in a period of time in which the temperature is relatively constant at 130–135°C. The color formation at this time is rapid and the reaction must be stopped abruptly to prevent the mass from turning to a resin. This is accomplished by adding water through a pipe in the cover directly onto the heated syrup. This not only cools but also dilutes the syrup. The syrup at about 90°C passes to a storage tank, from which it is fed to clarifying centrifugals. Some colors are further clarified by means of a filter press, precoated with filter aid. The clarified caramel color passes to a large storage tank. Uniform tinctorial power is achieved by decreasing or increasing the color strength of subsequent batches.

The composition of the catalyst varies according to the type of caramel being produced and also according to the ideas of the manufacturer. Needless to say, the exact composition of these formulas is a closely guarded secret. A beverage caramel must be "burnt" on the acid side. Starting with a sugar syrup with a pH in the range of 4.0 to 4.5, the pH decreases as the reaction proceeds. Thus a condition results wherein the pH may decrease to such an extent that the sugar mass will resinify. To prevent excess acidity from developing, the ammonia is added as ammonium sulfite or as ammonia and sodium sulfite. The pH is stabilized through the release of sulfurous acid to the atmosphere.

Closed-Kettle Process. The closed-kettle process resulted from research on caramel color which divided the process into two steps: the acid and heat treatment of the sugar syrup to polymerize the dextrose, and the addition of ammonia and/or salts, with a further heat treatment to produce the color. The kettles are jacketed, 6 ft in diameter, about 16 ft tall, with a capacity of about 2700 gal. The bottom is equipped with an outlet, and the top with a manhole, thermometer, pressure gage, vent pipe, and other pipes for the addition of water, ammonia, and compressed air. The kettles are stainless clad.

The production of a beverage color by the closed-kettle process is discussed in detail in a patent (4,9). This discussion includes a temperature graph and the various steps in a batch of caramel as related to temperature. Briefly, the kettle is loaded with 1600-1700 gal of 45° Bé sugar syrup (about 18,000 lb). Dilute sulfuric acid is then added to reduce the pH to the range of 1.2–1.5. The syrup is then heated to about 120°C for about 3 hr which reduces the DE of the sugar syrup to below 45. A predetermined quantity of anhydrous ammonia is then added and heat is applied to initiate the color reaction. When about half of the final tinctorial power has been obtained, an ammonia salt and sodium bisulfite are added. Heat is applied to increase the temperature of the syrup to the range of 132–135°C, venting the gas to maintain about 15 lb internal pressure.

When the desired tinctorial power has been obtained, the syrup is cooled by the addition of water and by passing cooling water through the jacket. The processing from this point on is the same as for the open-kettle process. Patents have been issued on the processing of caramel color to yield a more concentrated coloring and to reuse the unreacted sugars (2,8).

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W. R. FETZER
Consultant

CARBAMIC ACID

Carbamic acid, NH_2COOH, is the monoamide of carbonic acid; the diamide is the well-known compound urea (qv), $(NH_2)_2CO$, also called carbamide. Guanidine (qv), $HN=C(NH_2)_2$, may be regarded as the amidine of carbamic acid. Carbamic acid is not known in the free state but the acid chloride (chloroformamide, "urea chloride"), NH_2COCl, and salts and esters have been prepared. Ammonium carbamate, NH_2COONH_4, can be obtained as a white crystalline solid by reaction of dry carbon dioxide and ammonia; it is found in commercial ammonium carbonate (see Ammonium compounds). The best-known ester is the ethyl ester, $NH_2COOC_2H_5$, usually called urethan. Other esters of carbamic acid and of its N-substituted derivatives such as carbanilic acid, $C_6H_5NHCOOH$, are also called urethans (qv), somewhat loosely.

PHYSIOLOGY - Caramel and dextrin prepared by the action of dry heat on glucose and starch have the physiological qualities of structured compounds. Note from Mr. Paul Fournier, presented by Mr. Robert Courrier.

Glucose and starch are transformed by roasting into caramel and into dextrin possessing various physiological properties of structured compounds. When ingested by young rats, this caramel and dextrin increase the absorption and retention of calcium, favor the return of calcemia to normal levels, and encourage the rapid development of the cecum.

The term structured compounds is used to designate the numerous substances which bring about the following physiological reactions accompanying ingestion.

First, an osteogenic capacity which is manifested by a protective effect with respect to the resorption of the skeleton of the suckling rat and by the temporary increase in the speed of formation of the long bone of the young rat. This increase corresponds to an increase in the absorption and retention of calcium.

Second, a rapid return of calcemia to its normal level in a rat in which the calcium retention of the blood had been reduced by lack of calcium.

Third, a development of cecum which appears to be part of a more general phenomenon of hyperplasia of visceral organs.

Fourth, a urine composition characterized by a high content of various acids of the tricarboxylic cycle.

1

We indicated earlier why it became difficult to relate the essentially plastic properties of structured compounds to the initial activity of intestinal functions or to the existence of a metabolism peculiar to these compounds. Also, we are led to suppose as an hypothesis, that the action of structured compounds could be related to their poor utilization by the organism.

The action of dry heat on glucose and on starch leads to the production of caramel and dextrin, ill-defined substances which, because of the specificity of enzymes, have every chance of being less easily metabolized than those typically usable substances from which they come. The fact that caramel and dextrin are poorly metabolized is sufficient reason to consider them structured compounds, according to our hypothesis. Moreover, the presence of dextrin in a rickets-producing diet of a young rat increases the absorption of calcium. In the present work, caramel and dextrin are submitted to the first three tests of structured compound activity; calcium utilization, re-establishment of [normal] calcemia, and development of the cecum.

Experimental conditions: Glucose is caramelized by heat until the formation of a sticky brown paste which coagulates when cooled. The analytical characteristics of this caramel are as follows: a monhydrolized aqueous solution, having 88% of the rotation and 10% of the reducing power of the initial glucose; after acid hydrolysis, these values are raised to 94 and 91% respectively. Cornstarch is heated in an oven, at 190°C, for 5 hours. The ochre powder obtained, only slightly soluble

in water, furnished, through acid hydrolysis, a solution of which the
rotation and reducing powers were 94 and 78% respectively of those of
the initial starch. Enzymatic hydrolysis, achieved as is indicated in
the codex for a test for pancreatin, yields only 2% maltose from the
dextrin; under the same conditions, corn starch produced about 20% maltose.

First and second tests: Effects of caramel and dextrin on calcium
exchange and on the reestablishment of calcemia.--In an initial period of
18 days, 24 Wistar rats, weighing 62-74 g, were subjected to an "initial"
diet containing very little calcium (50 mg per 100 g), In the following
period, these rats were divided into 4 equal groups. Each diet described
in the table under the names: starch, dextrin, glucose, and caramel,
corresponds to one of the 4 groups. Calcium content in the four diets
is normal.

		Initial	Starch	Dextrin	Glucose	Caramel
	Crude casein	15	15	15	15	15
NB:"b	Peanut oil	8	8	8	8	8
can be	Cornstarch	72	70.5	45.5	58.5	58.5
corn, or	Other glycosidic compounds	0	0	23	12	12
grain	Dry yeast	3	3	3	3	3
*	Saline mixture	3	3	3	3	3
	Calcium carbonate ($CaCO_3$)	0	1.5	1.5	1.5	1.5
	Titanium oxide (O_2T_i)	0.5	0.5	0.5	0.5	0.5

* derived from Hubbel's mixture by suppression of calcium carbonate

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From the third to the fifth day of administration of these diets, each rat was put into an individual compartment which permitted separate collection of urine and feces. The calcium in the diet and the excrements was determined; the results of these analyses were used to calculate the calcium balance. When the rats had received their respective diets for 10 days, they were sacrificed, and their serum calcium was determined.

Third test: Effects of caramel and dextrin on the development of the cecum. Wistar rats weighing from 50 to 61 g were divided into three groups of 8. Those in the first group received a "starch" diet of the following composition: cornstarch, 73.5%; casein, 12%; peanut oil, 8%; saline mixture, 3%; yeast, 3%; O_2T_i (titanium oxide), 0.5%. Dextrin replaced starch in the diet of the rats in the second group. The diets of the rats of the two other groups differed from the first only in that 25% glucose or 25% caramel was substituted for the same proportion of starch. After two weeks of the experiment the rats were sacrificed. Their cecum was separated, emptied, cleaned, dried, and weighed. Note that for the same diet, the average weight of rats in each group is practically the same.

1st Test: calcium balance	Starch	Dextrin	Glucose	Caramel
Calcium (mg/day)				
Ingested	61.2	70	63	62.4
Absorbed	16.7	36.5	20	33.9
Urinary	1.3	1	0.5	0.4
Retained	15.4	35.5	19.5	33.5

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2nd test: serum calcium	Starch	Dextrin	Glucose	Caramel
Calcium (mg/liter)				
Average	88	128	91	118
Extremes	75,95	120,132	85,98	110,125
3rd test: Dry weight of cecum				
Average per group (mg)	67	152	73	192
Extremes	53,84	132,183	49,87	158,243

Results and conclusions. - The results grouped in the table show that dextrin and caramel have the properties of structured compounds. In the range of their ingestion, calcium absorption and retention are doubled. In the case of young rats whose serum calcium was at first drastically lowered through a calcium-deficient diet, calcemia was restored to normal much more rapidly if the calcium diet administered contained dextrin or caramel. Moreover, these two substances caused a rapid and strong cecal hypertrophy.

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PHYSIOLOGIE.   *Le caramel et la dextrine préparés par action de la chaleur
  sèche sur le glucose et l'amidon possèdent les qualités physiologiques des
  composés de structure.* Note (*) de M. PAUL FOURNIER (¹), présentée par
  M. Robert Courrier.

Le glucose et l'amidon sont respectivement transformés, par grillage, en caramel
et en dextrine dotés des diverses propriétés physiologiques des composés de struc-
ture. Ingérés par le jeune Rat, ce caramel et cette dextrine accroissent l'absorption
et la rétention calciques, favorisent le retour de la calcémie au niveau normal,
provoquent le rapide développement du caecum.

Par composés de structure, nous désignons les nombreux corps dont
l'ingestion s'accompagne des répercussions physiologiques suivantes :
   1° un pouvoir ostéogène qui se manifeste par un effet protecteur à
l'égard de la résorption du squelette de la Ratte allaitante et par un
accroissement temporaire de la vitesse de formation des os longs du
jeune Rat. A cet accroissement correspond une augmentation de l'absorp-
tion et de la rétention du calcium (²);
   2° un retour rapide de la calcémie à son niveau normal chez le Rat
dont la teneur du sang en calcium a été abaissée par carence calcique (³);
   3° un développement du caecum, développement qui semble s'inscrire
dans un phénomène plus général d'hyperplasie d'organes viscéraux (⁴);
   4° une composition des urines caractérisée par une forte teneur en divers
acides du cycle tricarboxylique (⁵).

   Nous avons dit pour quelles raisons il devient difficile de rapporter ces
propriétés essentiellement plastiques des composés de structure à l'acti-
vation des fermentations intestinales ou à l'existence d'un métabolisme
particulier à ces composés (⁶). Aussi sommes-nous conduit à supposer,
en hypothèse, que l'action des composés de structure pourrait être en
rapport avec leur mauvaise utilisation par l'organisme.

   L'action de la chaleur sèche sur le glucose et sur l'amidon conduit à
l'obtention de caramel, de dextrine, corps mal définis qui, du fait de
la spécificité des enzymes, ont toutes chances d'être moins aisément
métabolisés que les corps typiquement utilisables dont ils proviennent.
Selon notre hypothèse, il n'en faudrait pas davantage pour que le caramel
et la dextrine soient dotés des qualités des composés de structure. D'autant
que la présence de dextrine dans le régime rachitigène du jeune Rat amélio-
rerait l'absorption du calcium (⁶). Dans le présent travail, le caramel et
la dextrine sont soumis aux trois premiers tests de l'action des composés
de structure : utilisation calcique, rétablissement de la calcémie, déve-
loppement du caecum.

   CONDITIONS EXPÉRIMENTALES.   Le glucose est caramélisé par chauf-
fage jusqu'à l'obtention d'une pâte collante brune qui se prend en masse

par refroidissement. Les caractères analytiques de ce caramel sont les suivants : une solution aqueuse, non hydrolysée, possède 88 °/₀ du pouvoir rotatoire et 10 °/₀ du pouvoir réducteur du glucose initial; après hydrolyse acide, ces valeurs s'élèvent respectivement à 94 et 94 °/₀. De l'amidon de blé est chauffé au four, à 190°, pendant 5 h. La poudre ocrée obtenue, très peu soluble dans l'eau, fournit, par hydrolyse acide, une solution dont les pouvoirs rotatoire et réducteur représentent respectivement 94 et 78 °/₀ de ceux de l'amidon initial. Par hydrolyse enzymatique, réalisée comme il est indiqué au Codex pour l'essai de la pancréatine, cette dextrine ne libère que 2 °/₀ de maltose; dans les mêmes conditions, l'amidon de blé en fournit environ 20 °/₀.

1ᵉʳ et 2ᵉ tests : *Effets du caramel et de la dextrine sur les échanges calciques et sur le rétablissement de la calcémie.* — Dans une période initiale de 15 jours, 24 rats Wistar, de 62 à 74 g. sont soumis à un régime « initial » contenant très peu de calcium (50 mg pour 100 g). Dans la période suivante ces rats sont répartis en quatre lots égaux. A chaque lot correspond l'un des régimes décrits dans le tableau sous les noms : amidon, dextrine, glucose, caramel. La teneur en calcium de ces quatre régimes est normale.

	Initial.	Amidon.	Dextrine.	Glucose.	Caramel.
Caséine brute..............	15	15	15	15	15
Huile d'arachide...........	8	8	8	8	8
Amidon de blé.............	70	70.5	45.5	58.5	58.5
Autre composé glucidique ...	0	0	25	12	12
Levure sèche..............	3	3	3	3	3
Mélange salin (*)...........	3	3	3	3	3
CaCO₃....................	0	1.5	1.5	1.5	1.5
O₂Ti.....................	0.5	0.5	0.5	0.5	0.5

(*) Il dérive de celui de Hubbel (⁷) par suppression de CO₃Ca.

Du 3ᵉ au 5ᵉ jour d'administration de ces régimes, chaque rat est introduit dans un dispositif individuel qui permet de récolter séparément les urines et les fèces. Le calcium est dosé dans le régime et dans les excréments; les résultats de ces dosages servent au calcul des bilans calciques. Quand les rats ont reçu leurs régimes respectifs pendant 10 jours, ils sont sacrifiés; le calcium est dosé dans leur sérum.

3ᵉ test : *Effets du caramel et de la dextrine sur le développement du cæcum.* — Des rats Wistar de 50 à 61 g sont répartis en trois lots de 8. Ceux du premier lot reçoivent un régime « amidon » de composition centésimale suivante : amidon de blé, 73,5; caséine, 12; huile d'arachide, 8; mélange salin (⁷), 3; levure, 3; O₂Ti, 0.5. Dans le régime des rats du deuxième lot, la dextrine remplace l'amidon. Les régimes des rats des deux autres lots ne diffèrent du premier que par l'incorporation de 25 °/₀ de glucose ou de caramel à la place d'une même proportion d'amidon. Après deux semaines d'expérience, les rats sont sacrifiés. Leur cæcum est séparé, vidé,

nettoyé, séché et pesé. Notons que pour une même expérience le poids moyen des rats de chaque lot est pratiquement le même.

	Amidon.	Dextrine.	Glucose.	Caramel.
1er test : Bilans calciques.				
Calcium (mg/jour) :				
Ingéré................	61,2	70	63	62,4
Absorbé..............	16,7	36,5	30	33,9
Urinaire.............	1,3	1	0,5	0,4
Retenu..............	15,4	35,5	19,5	33,5
2e test : Calcium sérique.				
Calcium (mg/l) :				
Moyenne.............	88	128	91	118
Valeurs extrêmes.......	75; 95	120; 132	85; 98	110; 125
3e test : Poids sec du cæcum.				
Moyenne par lot (mg)...	67	152	73	192
Valeurs extrêmes.......	53; 84	132; 183	49; 87	158; 243

RÉSULTATS ET CONCLUSION. — Les résultats groupés dans le tableau montrent que la dextrine et le caramel possèdent les propriétés des composés de structure. Sous l'effet de leur ingestion, l'absorption et la rétention calciques sont doublées. Chez le jeune Rat dont la teneur du sérum en calcium a été d'abord très abaissée par un régime déficient en calcium, la calcémie redevient beaucoup plus rapidement normale si le régime calcique qui lui est offert contient de la dextrine ou du caramel. De plus, ces deux substances provoquent une rapide et forte hypertrophie cæcale.

(*) Séance du 22 juin 1959.
(¹) Avec la collaboration technique de Mme Monique Allez.
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(⁵) P. FOURNIER et A. DIGAUD, Bull. Soc. Chim. biol., 41, 1959, p. 173.
(⁶) O. BERGEIM, J. Biol. Chem., 70, 1926, p. 35.
(⁷) R. HUBBEL, L. MENDEL et A. WAKEMAN, J. Nutr., 14, 1937, p. 273.

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Caramel.

Caramel Color

Caramel is the amorphous dark brown material resulting from the controlled heat treatment of the following food grade carbohydrates:

Dextrose
Invert sugar
Lactose
Malt syrup
Molasses
Starch hydrolysates and fractions thereof
Sucrose

Not much is known about the nature of the caramelization of sugar; however, by control of the reaction, various grades are prepared with certain desirable properties.

The composition of caramel is complex and indefinite, forming a colloid in aqueous substrates. The colloids carry an electrical charge, the nature of which depends upon the method of manufacture. The isoelectric point of the caramel is quite important in various applications. At pH's above the isoelectric point, the caramel is negatively charged—at pH's below, it is positively charged. One of the major uses of caramel is in coloring carbonated beverages: colas and root beers. In order to prevent precipitation, the caramel must carry a strong negative charge and its isoelectric point should be at a pH of 2.0 or less.

Grades and Uses There are many tailor-made varieties of caramel, but most fall into the following types:

1. Acid proof caramel	For use in carbonated beverages and acidified solutions
2. Bakers and confectioners	A less refined grade for use in baked products—cookies, cakes, rye bread
3. Dry caramel	For use in dry mixes or where volume of liquid product is excessive.

Types 1 and 2 are dark-brown viscous liquids having a specific gravity in the range of 35° Be.

In most products, caramel has very good stability. It is very helpful in coloring of canned carbonated beverages in which the concentration of azo dye should be kept low. Addition of caramel helps to increase the depth of color; however, it must be carefully balanced with the certified color or "dirty" colors are produced. Caramel color is often used with certified colors in baked goods where dark chocolate shades are desired and which cannot be achieved without excessive use of certified color. One disadvantage of caramel color is its yellow-brown shade. For bakers' use, caramel products are available to which Red FD&C Colors have been added to give a more chocolate color. From a cost standpoint, caramel color is quite economical.

CARAMEL - GERMAN TRANSLATION

THE MODE OF ACTION OF CARAMEL IN NORMAL AND DIABETIC PEOPLE. STUDIES

WITH LEVOGLUCOSAN

Prof. E. Grafe and Cand. Med. Erica von Schroder
·(Rostock Medical Polyclinic)

Whereas the therapeutic use of caramel in diabetics is well established

today according to confirming data of all previous observers (1), the

mechanism of its favorable effect on glucosuria, acidosis, and protein

metabolism is still unknown.

The only established fact is that caramel undergoes oxidation in the

organism, but the way in which it is decomposed is still completely unknown.

Not even the form in which it is resorbed in the intestine has been established,

since enzymes and the intestinal bacteria investigated so far are unable to

break down caramel in the test tube (Grafe) (2). In this connection, basic

differences in the behavior of the normal and diabetic organism could not be

detected.

Further clarification of biological and physiological-chemical problems

to be considered here is not possible until something is known about the

chemical nature of the starting material. However, it is precisely here that

extradordinary difficulties arise, since the caramel formed by roasting (browning)

(1) Literature reviewed by E.Grafe in "Caramel in Diabetes Therapy",
     Fortschritte der ges. Medizin, publ. by Th. Brugsch, No.2, 1924
(2) Deutsch. Arch. f. Klin. Med. 116, 437, 1914

of pure sugar is not a pure (uniform) product, but rather a mixture of widely differing substances, standing somewhere in the middle between unchanged starting material and amorphous charcoal.

Gelis (3) attempted to isolate from this mixture several main components with varying O_2 - content and a high molecular weight. He thus differentiated three products:

1) Caramelan: 1. Caramelan: $6C_{12}O_{22}O_{11}-12H_2O=6C_{12}H_{18}O_9$

2. Caramelen: $6C_{12}H_{22}O_{11}-18H_2O=2C_{36}H_{48}O_{24}$

3. Caramelin: $6C_{12}H_{22}O_{11}-27H_2O=3C_{24}H_{26}O_{18}$

Gelis introduced at that time the name glucosans for the grape sugar (d-glucose) anhydrides, while he used the name levoglucosane to designate the corresponding cane sugar derivatives. The latter were then identified much later by Tanret (1) as a component of most gluosides.

In all cases, it was assumed that inner anhydrides of the sugar were formed as a result of a lesser or greater loss of water. Only in recent years did Swiss chemists, primarily Pictet and Karrer, together with their various associates, succeed in obtaining and describing uniform and well-defined pure compounds by roasting (browning) pure carbohydrates.

Since it is advisable, in our study of the biological action of caramel, to start with these so-called glucosans, a brief chemical description of these compounds should be given before we describe our own experiments; for all details, the reader should refer to the latest and numerous original studies carried out in the laboratories of Pictet and Karrer(2).

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(3) Compt. rend. 48, 1062 (1859); 51,331(1860); Arch.de Chim.(3),57,254

(1) Ber.[3],11,949(1894); Compt.rend.de P'Acad.d.sc. 119, 158

(2) Mostly published in Helv.Chim.Acta. Literature reviewed by Zempleu, in Abder halden's Handb. d. biol. Arbeits   Abt.I,T.5,H.1,Lief.52,S.549 ff,1922

For our purposes, the compounds of greatest interest are
α- glucosan and levoglucosan. The former is of particular interest, since
it was basically obtained in the same manner by Pictet and Castan (3), as the firm
Merck produced caramose in 1913 at the suggestion of Grafe.

When pure grape sugar is heated at 150-155°C under 15 mm mercury pressure
and the unchanged glucose is removed by brief warming in absolute alcohol,
α-glucosan is obtained in 92% yield, in the form of colorless leaflets
(m.p. 108-109°), following recrystallization from absolute methyl alcohol. The
compound is very hygroscopic, but is not converted back into glucose; it is
highly soluble in water, somewhat less in methyl alcohol and acetic acid, and
practically insoluble in alcohol. Its specific rotation is $[\alpha]_D^{21} = + 69.4$ and
$69.79°$. The compound reduces an alkaline copper sulfate solution, but does
not give a red color with Schiff's solution. Upon boiling in water, it is
converted into α-glucose. Its taste, as in caramel, is distinctly bitter.

A series of compounds was prepared, but these are of no interest here.

When pure cellulose or starch is subjected to the same treatment, at the
same pressure but slightly higher temperature (200-300°), levoglucosan is
obtained, but in a considerably smaller yield, according to Pictet and Sarasin(1).
The compound forms crystals, melting at 179.5°, is readily soluble in water,
acetone and acetoacetic acid, but almost insoluble in other organic solvents.
The specific rotation is $[\alpha]_D = 67.25°$. The compound decomposes slightly
during distillation under normal pressure, but is in general quite stable. It

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(3) Helv. Chim.Acta 3, 640(1920)(abstr.in Chem. Zentralbl.III, 879,1920
     4,319 (1921)

(1) Compt. rend. 166, 38(1918); Helv.Chim.Acta 1, 87(1918)(Abstr.in Chem.
     Zentralbl.II, 710(1918); cf. also Pictet and Cramer, Helv.Chim.Acta 3,
     640 (1920) (abstr. in Chem. Zentralbl.III, 878 (1920).

is not broken down by emulsin, maltase and yeast, and β-glucose
is formed under the action of dilute mineral acids. The compound has lost
its sugar character to a great extent, and does not reduce Fehling's solution
any longer. It is not oxidized by potassium permanganate and bromine. The
compound has a peculiar taste, which is both bitter and sweet at the same
time. Its caloric value is 4181, as compared to 3743 for glucose. By studying
suitable compounds, Pictet and Reichel (2) have been able in recent years to
establish a definite structural formula for levoglucosan:

$$
\begin{array}{c}
\text{C--H} \\
\text{H--C--OH} \\
\text{OH--C--H} \\
\text{H--C} \\
\text{H--COH} \\
\text{CH}_2
\end{array}
$$
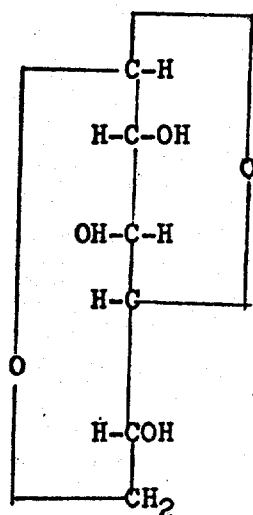
Karrer and Smirnoff (1) have confirmed this formula and have suggested
the following space formula:
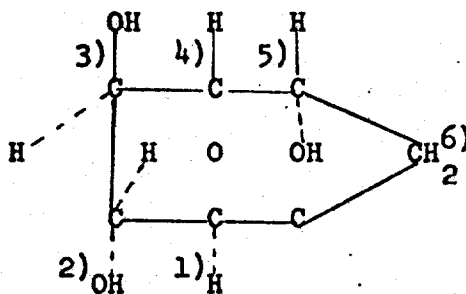
(2) Helv. Chim. Acta 6, 617 (1923)
(1) Helv. Chim. Acta 5, 124 (1922)

Thus, levoglucosan is a well-defined substance which has been fully characterized from a chemical standpoint. As Pictet and his associates have primarily shown, glucosans yield polymerization products under the action of zinc chloride. Thus, A. and I. Pictet (2), as well as A. Pictet and I.H. Ross (3) have prepared polylevoglucosans (di-,tetra-,hexa-, and octolevoglucosan), which are very similar to dextrins, but can be differentiated from the latter by means of chemical reactions. Analogous compounds can also be prepared from ⍺-glucosan(4).

As far as we know, the biological effect of all these substances has not yet been investigated. In view of the quite similar process by which these compounds are formed, there can be no doubt that the roasting (browning) of sugar and other carbohydrates yields glucosans. Since simple sugars yield mostly simple compounds and starch mostly high-molecular polymerization products, it appears highly desirable to undertake a study of the biological action of glucosans, in order to clarify the effect exerted by caramel in the normal and diabetic organism.

In view of the large number of questions which arise in this connection, one of us agreed to divide the work facing us with Dr. Kerb (Freiberg), who is greatly interested in the same problem, in such a way that our work will be concerned with levoglucosan, while Dr. Kerb will report the results of his analogous studies of ⍺-glucosans elsewhere (5).

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(2) Compt. rend. 173, 258 (1921)- Abstr. in Chem. Zentralbl.III, 822,(1921)

(3) (Ibid. 174, 1113 (1922) - Abstr. in Chem. Zentrabl. II, 346 (1922)

(4) A and I. Pictet, Compt. rend. 173, 158 (1921), Helv. Chim. Acta 6, 617,(1923)

(5) Biochem. Zeitschr. 144, 60 (1924)

In this article (see note), we shall attempt first to answer the following questions:

(1) What changes does levoglucosan undergo in the gastrointestinal tract?

(2) Can it be detected again in the urine?

(3) How does it affect glucosuria and acidosis in diabetic humans and animals?

(4) Does it cause changes in the blood sugar curve of normal and diabetic people?

NOTE: A brief report of this work was given at the October 1923 session of the Medical Society in Rostock and was also published in Klin. Wochenschr. 2, No. 49, 1923

We wish to express here our deep gratitude to Dr. Dohrn, of the Schering Chemical Plant in Berlin, for providing us cost-free with a sufficient amount of levoglucosan for our purposes.

We tested the effect of digestive enzymes in test tube experiments, using normal and artificial digestive juices. The polarimetric, analytical fermentation and reducing behavior of these juices was studied continuously and side-by-side according to the standard methods; Benedict's process was selected by us as the reduction method.

We found at first that a 6.2% aqueous levoglucosan solution, after addition of a small amount of toluene, did not exhibit any changes even after a 5-day storage in an incubator. The fermentation and reduction samples proved to be continuously negative. The levorotation remained unchanged. When natural gastric juice was used, the presence of diastase and small amounts of starch proved to be disturbing; on the other hand, when artificial

gastric juice was used, which was brought to a normal acid and enzyme content by addition of 0.1 N HCL and pepsin, the reduction and fermentation samples remained continuously negative, as shown in the following experiment:

TABLE I

10 ml artificial gastric juice, containing 0.62 g levoglucosan + 0.5 ml toluene

Testing Time	Reducing Action	Fermenting Capacity	Polarimetrically
Freshly used			
After 24 hours	0	0	-7.6%
After 48 hours	0	0	-7.6%
After 72 hours	0	0	-7.7%
After 96 hours	0	0	-7.7%
After 120 hours	0	0	-7.8%

Klin. Wochenschr. 2, Nr. 49, 1923

The slight increase in the levorotation is due to water evaporation.

The same results were obtained in tests carried out with the strongly active pancreas dispersion (Krause) and pure cultures of coli bacteria, which were kindly made available to us by the local Hygiene Institute (examples of this work are given in the inaugural dissertation of Miss von Schroeder (1).

It is not unlikely that other intestinal bacteria, particularly anaerobic bacteria, might exhibit a different behavior in this case; special tests should be carried out in this direction.

Caramel and levoglucosan show a completely parallel behavior in regard to their resistance to attack by gastrointestinal enzymes.

_____

(1) Study of Caramel Action (tests with levoglucosan), Rostock, 1923

Insufficient amounts of the very expensive preparation were available to us for utilization tests in feces, which should be carried out by all means. In view of the extremely high water solubility and the low molecular weight of levoglucosan, it seems a priori highly unlikely that this compound would exhibit a poor or incomplete resorption. Also the fact (which will be discussed shortly) that a levorotation in the urine can be detected only during the first 2 hours following ingestion provides a strong argument against such a possibility.

In order to test excretion conditions in the urine, healthy fasting people were given 250 g coffee with 20 g levoglucosan; the urine was then tested at hourly intervals for a reducing, fermenting and polarimetric action.

The following example of a test carried out on a patient with Lues cerebrospinalis (but with a normal metabolism) may serve as an illustration:

TABLE 2

Time 4.IX.1923	Food Intake	Urine Amount	Reducing Power of Urine	Ferment- ing capac- ity of urine	Polarim- etric study	Levoglu- cosan ex- creted, g/hr	Total Levoglucosan, g
6-7 PM	Fasting	88 ml	0	0	0	–	–
7-8 PM	250 coffee with 20 g levoglucosan	128 ml	0	0	-0.8%	=8.803	1.070
8-9 PM	No food	170 ml	0	0	-0.2%	=0.267	
9-19 PM	intake	--	0	0	0		

Changes were observed only in the polarimetric study. During the first 2 hours of levoglucosan ingestion, a levorotation was observed, which was more marked only during the first hour; this proves that resorption of the preparation apparently takes place at an extremely rapid rate. If we calculate, on the basis of the specific rotation of levoglucosan, the amount of the compound which was excreted unchanged, then we get an amount

equal to 1.070 g (approx. 5% of the starting material), which must be considered as being very small. That this amount really represents levoglucosan can be easily demonstrated by detection of reducing glucose after boiling with dilute sulfuric acid.

Of special interest, of course, was the action of levoglucosan in the diabetic organism. Prof. Curschmann was kind enough to allow us to carry out such tests on patients transferred from the polyclinic to the inner clinic, and we are grateful for this possibility; other tests were carried out on private patients under close clinical supervision.

The outcome of all tests was always the same. An increase in glucosuria was never observed, 5-10% levoglucosan was detected in the urine, and if acidosis was present it was favorably influenced by levoglucosan intake. The following example illustrates this fact.

The patient Fl. (see Table III) had diabetes of medium severity; even after several days on a vegetable-rich diet he was not completely free of sugar and acidosis. In this patient, a comparison was made of sugar and acetone body excretion on 2 consecutive days over 3-hour periods with and without addition of 30 g levoglucosan to a basic diet of 100 g spinach, 50 g lard and 250 g coffee. The sugar excretion, determined by titration according to Benedict, was identical during both comparison periods, so that sugar formation from levoglucosan was excluded. On the other hand, the effect on acidosis is very characteristic: under the action of levoglucosan, acidosis dropped to 1/10 of its original value (from 0.48 to 0.047 g).

In regard to the action of levoglucosan in a patient with a slightly less severe diabetes, but with a very low tolerance, the characteristic course during 4-hour periods is shown in Table IV. Here also no trace of sugar was detected, although a noticeable levorotation was observed, which also

only during the first hour was higher by about 0.8% and, calculated on
the basis of levoglucosan, amounted to only 0.878 g, thus being equal to
less than 4.5% of the ingested amount of levoglucosan. (See Table III and
Table IV.)

Finally, we wish to report a series of observations performed on an
acute diabetic (Table V), who could be made free of sugar and acidosis only
with insulin. This diabetic, on the third day of a vegetable diet, still
excreted 30.8 g sugar and 4.74 g acetone. On the next day, the patient
received in his previous diet an addition of 30 g levoglucosan, which reduced
the sugar excretion quite moderately (to 24.15 g) but the acidosis quite
significantly, namely to less than 1/2 the original value (1.99 g).
(See Table V.)

This day was broken down into individual segments. Here, we could
observe that sugar excretion (calculated per hour) was even somewhat lower
(1.94 g), during the hours immediately following levoglucosan intake, than
previously in the fasting stage (2.03 g). The favorable effect on acidosis
became clearly apparent only during later hours.

Table VI, for which we are indebted to Dr. Otto Martiensen, shows that
a phlorizin-poisoned (diabetic) dog exhibits the same behavior as a diabetic.
(See Table VI.)

The sugar excretion remains unchanged from a fermentation analysis
standpoint, while the figures even show a decrease, when measured according
to Benedict. Particularly clear is the action exerted on protein metabolism,
which drops to less than 50% of the value on the previous day; here also
is a complete analogy with the action of caramel.

In individual cases, the effect exerted on blood sugar (according to Benedict) was also tested. As the two examples in Table VII show, following 20 g levoglucosan intake the blood sugar content is not increased but rather drops noticeably in the following hours. In this respect, there is a basic difference in comparison with caramel; the latter, according to studies of Magin and Turban [Deut.Arch. Klin. Med. 143 97-105 (1923)], exhibits an increase in blood sugar values, although this increase is temporary and not clearly marked. We must keep in mind, however, that even Merck's caramose may contain up to 10% reducing substances, whereas levoglucosan does not have any reducing properties at all.

If we summarize the previously investigated behavior of levoglucosan in the normal and diabetic organism, we find that enzymes present in the gastro-intestinal tract are unable to split this sugar anhydride; approximately 5-10% shows up in the urine; the compound has no glucosuric action; the blood sugar content is even somewhat reduced, whereas acidosis is very favorably affected and protein combustion is limited.

Thus, in all important points there is an extensive similarity in the biological action of caramel and levoglucosan; the latter, however, exhibits a clearer and definite effect, since it is a pure compound.

According to a written communication of Kerb (l.c.), who reports his findings elsewhere, α-glucosan behaves in exactly the same way as levoglucosan.

According to our studies, there is no doubt that pure glycosans, whose biological action has been tested for the first time, are indeed the main carriers of the favorable effect exerted by browned carbohydrates on the diabetic organism. To clarify the mechanism of the action of glucosans is the task of future investigations.

Apparently, there are two main possibilities of explaining this action. It is possible that levoglucosan either is involved somehow, perhaps through uptake of water, in the usual carbohydrate metabolism, possibly by stimulating glycogen formation, which could account for the peculiar behavior of blood sugar; or, the complex, but very resistant carbohydrate ring is split by oxidation and broken down in a still unknown manner, perhaps quite different from the way in which carbohydrates are usually broken down.

Thus, many interesting questions arise in this new field of carbohydrate physiology. The present investigations provide a secure basis for further constructive work.

In view of the particularly favorable action of levoglucosan in diabetes and its sweet taste, this sugar anhydride would be a very suitable sugar substitute, both from a therapeutic and dietetic standpoint; unfortunately, its use cannot yet be considered in view of the high manufacturing costs.

DATE	TIME	FOOD INTAKE	URINE AMOUNT	SUGAR/HR ACCORDING TO BENEDICT	TOTAL SUGAR/ TEST	SUGAR/HR- POLARIM- ETRICALLY DETERMINED	SUGAR PER TEST	ACETONE, QUALITA- TIVE TEST	ACETO- ACETIC ACID, QUALITA- TIVE TEST	TOTAL ACETONE IN G, PER TEST
26.VII 1923	10-11 m	around 9.45 50 g lard 100 g spin- ach 250 g coffee	110 ml	0.63 g	1.065 g	0.3%=0.33 g	0.51 g	+	+	0.48 g
	11-12	--	60 ml	0.435 g		0.3%=0.18 g				
	12-1	--	230 ml	0		0		heavy	+ -	
27.VII 1923	7-8	around 6:45 50 g lard 100 g spin- ach 250 +30	90 ml	0.315	1.075 g	0.2%=0.18 g	0.53 g	+	heavy +	0.047 g
	8-9	--	100 ml	0.425		0.2%=0.20		heavy +	heavy +	
	9-10 hr	--	75 ml	0.335		0.2%=0.15		heavy +	heavy +	

TEST PERIOD NO.	TIME	URINE AMOUNT ml	MYLANDER'S TEST RESULT	ACETONE TEST RESULT	POLARIMETRIC BEHAVIOR	EXCRETED LEVOGLUCOSAN CALCULATED FROM THE LEVO-ROTATION
1st hour prior to levoglucosan	9:30-10 h	30 ml	negative	negative	0%	
2nd hour immediately after injection of 26 g levo glucosan	10-11 h	110 ml	negative	negative	-0.8%	=0.690 g =0.878 g
3rd hour 2/hr after levoglucosan	11-12 h	120 ml	negative	negative	-0.2%	=0.188 g
4th hour 3/hr after levoglucosan	12-1 h	130 ml	negative	negative	0.0%	

TABLE V

DATE 1923	TEST INTERVAL	DIET COMPOSITION	CARBOHYDRATE CONTENT	URINE AMOUNT	SUGAR TITRATED	SUGAR FERMENTATION ANALYSIS	SUGAR POLARIM- ETRICALLY	ACETRIC ACID ACTION	TOTAL ACETONE
19-20X	3rd day of treatment	.30 g fat 2 eggs 30 g cheese 600 g veg. (asparagus, spinach) 1 l broth =12 cal/kg	30 g	2700 ml	30.8 g	--	--	++	4.74 g
20.21X	4th day of treatment	250 ml coffee	0	114 ml	4.4 g =2.03 g/hr	4.5 g	4.3 g	++	0.19 g
	9:25-12:10	250 g tea with 30 g levoglu- cosan (125 cal)	30 g	162 "	5.35 g =1.94 g/hr	6.48 g	2.9 g	++	0.3 g
	12:10-1:10	--	--	48 "	1.40 g	1.49 g	1.4 g	+	0.3 g
	1:10-8:10 on the following morning	30 g fat 2 eggs, 30 g cheese 600 g (asparagus, spinach) 1 l broth	30 g	1000 "	1.30 g	24.0 g?	12.0 g	+	1.4 g
	Whole day	cf. prev- iously	60 g	1324 "	24.15 g	--	20.6 g	+	1.99 g

SPECIAL - The yeast used was not tested for self-
fermentation. Perhaps this fact explains
the high value obtained (too high)

TABLE VI

DATE	FOOD INTAKE	WEIGHT kg	URINE AMOUNT	G SUGAR FERMENTA- TION ANALYSIS	G SUGAR BENEDICT	gN	D N	REMARKS
19.-20. VI	Hunger rings per probe(?)	5.85	570 ml	21.6	19.38	3.537	6.1	1 g Phlori- zin in oil
20.-21 VI	"	5.80	804 "	16.9	14.48	2.564	6.6	
21.-22	"	5.60	400 "	16.0	10.40?	3.035	5.2	1 g phlori- zin in oil
22.-23. VI	"	5.42	920 "	17.48	23.02	4.278	4.1	
23.-24.	" +8g levoglucosan	5.25	600 "	17.35	18.6	1.994	8.7	1 g phlori- zin in oil

TABLE VII

TIME	BLOOD SUGAR FIGURE IN B	BLOOD SUGAR FIGURE IN Fl	BLOOD SUGAR FIGURE IN DR. G
Before Levoglucosan	0.135%	0.18%	0.218
3/4 hr after after 20 g Levoglucosan	0.08 %	0.18%	0.20
2 hr after Levoglucosan	0.12%	0.125%	0.21
4 hr after Levoglucosan	0.10%	0.15%	--

Aus der Medizinischen Poliklinik zu Rostock.

Zur Kenntnis der Wirkungsweise des Caramels im gesunden und diabetischen Organismus.

(Versuche mit Lävoglucosan.)

Von

Professor E. Grafe und cand. med. Erica von Schröder.

Während die therapeutische Brauchbarkeit des Caramels bei Diabetikern nach den übereinstimmenden Angaben aller bisherigen Beobachter[1] wohl heute sichergestellt ist, liegt der Mechanismus der günstigen Wirkung auf Glycosurie, Acidose und Eiweißumsatz noch in tiefes Dunkel gehüllt.

Fest steht nur, daß es im Organismus zur Oxydation kommt, aber die Art des Abbaus ist noch völlig unbekannt. Nicht einmal die Form, in der es vom Darm resorbiert wird, steht fest, da die Fermente und die daraufhin bisher untersuchten Darmbakterien es im Reagenzglas nicht zu spalten vermögen (Grafe).[2] Dabei konnten prinzipielle Unterschiede im Verhalten des normalen und diabetischen Organismus bisher nicht festgestellt werden.

Eine weitere Klärung der biologischen und physiologisch-chemischen Fragen, die hier auftreten, ist nicht eher möglich, als bis das Ausgangsmaterial einigermaßen chemisch bekannt ist. Aber gerade in dieser Richtung türmen sich außerordentliche Schwierigkeiten auf, denn das bei der Röstung von reinem Zucker entstehende Caramel ist kein reiner Körper, sondern ein Gemisch außerordentlich verschiedener Substanzen, die in der Mitte zwischen unverändertem Ausgangsmaterial und amorpher Kohle stehen.

Schon Gélis[3] versuchte aus diesem Gemische mehrere Haupt-

1) Lit. bei E. Grafe. Das Caramel in der Diabetestherapie, Fortschritte der ges. Medizin, herausgeg. von Th. Brugsch, H. 2, 1924.

2) Deutsches Arch. f. klin. Med. 116, 437, 1914.

3) Compt. rend. 48, 1062, 1859 u. 51, 331, 1860 und Arch. de Chim. (3), 57, 254.

bestandteile mit wechselndem O_2-Gehalt und hohem Molekulargewicht zu isolieren.

Er unterschied so 3 Produkte:

1. Caramelan: $6C_{12}O_{22}O_{11} - 12H_2O = 6H_{12}H_{18}O_9$
2. Caramelen: $6C_{12}H_{22}O_{11} - 18H_2O = 2C_{36}H_{48}O_{24}$
3. Caramelin: $6H_{12}H_{22}O_{11} - 27H_2O = 3C_{24}H_{26}O_{13}$.

Für die Traubenzuckeranhydride führte Gélis damals schon den Namen Glycosane ein, die entsprechenden Rohrzuckerderivate nannte er Lävoglucosane. Letztere wurde dann viel später von Tanret[1] als Bestandteil der meisten Glucoside erkannt.

In allen Fällen würden also durch mehr oder weniger großen Austritt von Wasser innere Anhydride des Zuckers entstehen. Erst in den letzten Jahren ist es schweizer Chemikern, vor allem Pictet und Karrer mit ihren verschiedenen Mitarbeitern, gelungen, bei der Röstung von reinen Kohlehydraten einheitliche, wohl definierte reine Substanzen zu gewinnen und zu charakterisieren.

Da es sich für das Studium der biologischen Wirkung des Caramels empfiehlt, von diesen sog. Glycosanen auszugehen, so muß der Mitteilung unserer eigenen Versuche eine kurze chemische Charakteristik dieser Stoffe vorausgeschickt werden, bezüglich aller Einzelheiten sei auf die neueren zahlreichen Originalarbeiten der Laboratorien von Pictet und Karrer[2] verwiesen.

Die beiden für unsere Zwecke am meisten interessierenden Substanzen sind das α-Glucosan und das Lävoglucosan. Das erstere hat ein um so größeres Interesse, als es im Prinzip in der gleichen Weise von Pictet und Castan[3] gewonnen wurde, wie die Firma Merck auf Grafe's Veranlassung 1913 die Caramose herstellte.

Erhitzt man reinen Traubenzucker unter 15 mm Hg-Druck auf 150—155° und entfernt die unveränderte Glucose durch kurzes Erwärmen in absolutem Alkohol, so gewinnt man nach Auskristallisieren aus absolutem Methylalkohol mit 92 % Ausbeute in farblosen Blättchen mit einem Schmelzpunkt von 108—109° das α-Glucosan. Die Substanz ist sehr hygroskopisch, ohne sich jedoch dabei in Glucose zurück zu verwandeln.

Die Löslichkeit in Wasser ist groß, etwas geringer in Methylalkohol und Essigsäure, kaum vorhanden bei Alkohol

1) Ber. [3] 11, 949, 1891 und Compt. rend. de l'acad. d. sc. 119, 158.

2) Meist erschienen in den Helv. chim. act. Lit. bei Zemplen, Abderhalden's Handb. d. biol. Arbeitsm. Abt. I, T. 5, H. 1, Lief. 52, S. 549 ff., 1922.

3) Helv. chim. act. 3, 640, 1920 (ref. Chem. Zentralbl. III, 879, 1920) und 4, 319, 1921.

$[\alpha]_D^{21} = + 69,4$ bzw. $69,79°$.

Es reduziert alkalische Kupfersulfatlösung, rötet aber fuchsinschweflige Säure nicht. Beim Kochen in Wasser geht es in α-Glucose über. Der Geschmack ist wie beim Caramel ausgesprochen bitter.

Es sind eine Reihe von Verbindungen hergestellt, die aber hier nicht interessieren.

Unterwirft man bei gleichem Druck, aber etwas höheren Temperaturen (200—300°) reine Cellulose oder Stärke dem gleichen Verfahren, so erhält man mit allerdings erheblich geringerer Ausbeute nach Pictet und Sarasin[1] das Lävoglucosan. Die Kristalle schmelzen bei 179,5°. Sie sind leicht löslich in Wasser, Aceton und Acetessigsäure, dagegen fast unlöslich in anderen organischen Lösungsmitteln. Die spezifische Drehung $[\alpha]_D = 67,25°$. Die Verbindung zersetzt sich zwar etwas beim Destillieren unter gewöhnlichem Druck, ist aber im ganzen sehr beständig. Emulsin, Maltase und Hefe spalten nicht, unter Einwirkung verdünnter Mineralsäuren entsteht β-Glucose. Der Zuckercharakter ist weitgehend verloren gegangen. So wird Fehling'sche Lösung nicht mehr reduziert. Durch Kaliumpermanganat und Brom tritt keine Oxydation ein. Eigentümlich ist der Geschmack, der zugleich bitter und süß ist. Der Brennwert ist 4181 gegenüber 3743 bei Glucose. Durch das Studium geeigneter Verbindungen ist es Pictet und Reichel[2] im letzten Jahre auch gelungen, die Konstitutionsformel definitiv festzustellen:
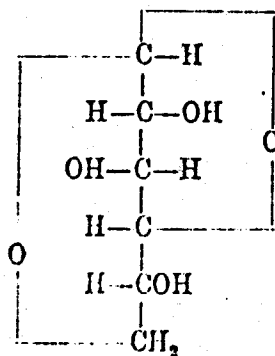
1) Compt. rend. 166, 38, 1918 u. Helv. chim. act. 1, 87, 1918 (ref. Chem. Zentralbl. II, 710, 1918) vgl. auch Pictet u. Cramer, Helv. chim. act. 3, 640, 1920 (ref. Chem. Zentralbl. III, 878, 1920).

2) Helv. Chim. act. 6, 617, 1923.

Karrer und Smirnoff haben sie bestätigt und sind für folgende Raumformel eingetreten:
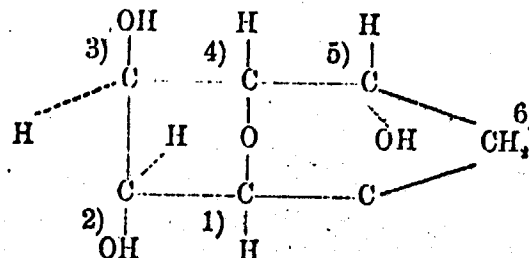
Wir haben also im Lävoglucosan einen chemisch in jeder Beziehung wohl charakterisierten und definierten Körper vor uns. Wie vor allem Pictet und seine Mitarbeiter zeigten, entstehen unter Einwirkung von Zinkchlorid aus den Glucosanen Polymerisationsprodukte. So sind von A. und I. Pictet[2] sowie A. Pictet und I. H. Roß[3] Polylävoglucosane hergestellt (Di-, tetra-, Hexa- und Octolävoglucosan). Sie haben große Ähnlichkeit mit Dextrinen, lassen sich aber durch chemische Reaktionen von ihnen unterscheiden. Analoge Verbindungen gibt es beim α-Glucosan.[4]

Die biologische Wirkung aller dieser Substanzen ist unseres Wissens bisher noch nicht weiter erforscht. Da es bei dem ganz ähnlichen Entstehungsvorgang keinem Zweifel unterliegen kann, daß bei der Röstung von Zucker und anderen Kohlehydraten Glucosane entstehen, beim einfachen Zucker wohl mehr die einfachen Verbindungen, bei der Stärke wohl mehr die hochmolekularen Polymerisationsprodukte, so scheint es zur Klärung der Caramelwirkung im normalen und diabetischen Organismus dringend wünschenswert, das Studium der biologischen Wirkung der Glucosane in Angriff zu nehmen.

Bei der großen Menge der sich hier erhebenden Fragen hat der eine von uns mit Herrn Dr. Kerb-Freiburg, der sich für die gleichen Probleme lebhaft interessiert, in der Weise eine Arbeitsteilung verabredet, daß wir selbst uns mit dem Lävoglucosan beschäftigen, während Herr Kerb über seine analogen Studien über die α-Glucosane an anderer Stelle berichten wird.[5]

1) Helv. chim. act. 5, 124, 1922.

2) Compt. rend. 173, 258, 1921 (ref. Chem. Zentralbl. III, 822, 1921).

3) Ebenda 174, 1113, 1922 (ref. Chem. Zentralbl. II, 346, 1922).

4) A. u. I. Pictet, Compt. rend. 173, 158, 1921 und Helv. Chim. act. 6, 617, 1923.

5) Biochem. Zeitschr. 111, 60, 1924.

In der vorliegenden Mitteilung[1]) sollen zunächst folgende Fragen entschieden werden:

1. welche Veränderungen erleidet das Lävoglucosan im Magendarmkanal?

2. läßt es sich im Harn wiederfinden?

3. wie beeinflußt es beim diabetischen Menschen und Tier Glycosurie und Acidose?

4. verändert es bei Gesunden und Diabetikern die Blutzuckerkurve?

Herr Dr. Dohrn von der chemischen Fabrik Schering-Berlin, hatte die große Freundlichkeit, für die wir ihm zu großem Danke verpflichtet sind, uns für unsere Zwecke Lävoglucosan in ausreichender Menge kostenlos herzustellen.

Den Einfluß der Verdauungfermente prüften wir in Reagenzglasversuchen mit normalen und künstlichen Verdauungssäften. Nebeneinander wurden fortlaufend polarimetrisches, gärungsanalytisches und reduzierendes Verhalten nach den üblichen Methoden verfolgt, als Reduktionsmethode wählten wir das Verfahren von Benedict.

Zunächst zeigte sich, daß eine 6,2%ige wäßrige Lävoglucosanlösung selbst nach 5tägigen Aufenthalt im Brutschrank nach Zusatz einer kleinen Toluolmenge keine Veränderungen zeigte. Die Gärungs- und Reduktionsproben fielen dauernd negativ aus. Die Linksdrehung blieb unverändert.

Bei Verwendung von natürlichem Magensaft störte das Vorhandensein von Diastase und kleinen Stärkemengen, dagegen blieben bei Verwendung von künstlichen Magensaft, der durch $\frac{n}{10}$ Salzsäure und Pepsinzusatz auf normalen Säure- und Fermentgehalt gebracht wurde, die Reduktions- und Gärungsproben dauernd negativ, wie folgender Versuch zeigt:

Tabelle I.

10 ccm künstlicher Magensaft, enthaltend 0,62 g Lävoglucosan + 0,5 ccm Toluol.

Zeit der Prüfung	reduzierende Wirkung	Gärungs-vermögen	polarimetrisch
frisch angesetzt	0	0	— 7,6 %
nach 1 mal 24 Stunden	0	0	— 7,6 %
nach 2 mal 24 Stunden	0	0	— 7,7 %
nach 3 mal 24 Stunden	0	0	— 7,7 %
nach 4 mal 24 Stunden	0	0	— 7,7 %
nach 5 mal 24 Stunden	0	0	— 7,8 %

1) Ein kurzer Bericht erfolgte in der Oktobersitzung 1923 der Mediz. Gesellsch. in Rostock sowie Klin. Wochenschr. 2, Nr. 49, 1923.

Die geringe Zunahme der Linksdrehung ist durch Wasserverdampfung bedingt.

Genau so fielen die Versuche mit dem stark wirksamen Pankreasdispert (Krause) sowie mit Colibakterienreinkulturen, die uns das hiesige hygienische Institut in liebenswürdiger Weise zur Verfügung stellte, aus. (Beispiele in der Inauguraldissertation von Frl. von Schröder[1]).)

Es ist nicht ausgeschlossen, daß andere Darmbakterien, insbesondere die Anaerobier, sich da anders verhalten, in dieser Richtung wären noch besondere Versuche auszuführen.

Hinsichtlich der Unangreifbarkeit durch die Magendarmfermente ist der Parallelismus zwischen Caramel und Lävoglucosan ein vollkommener.

Für Ausnutzungsversuche im Stuhl, die unbedingt noch angestellt werden müssen, standen uns nicht genügende Mengen des sehr teuren Präparates zur Verfügung. Bei der außerordentlichen Wasserlöslichkeit und dem niedrigen Molekulargewicht des Lävoglucosans ist es aber a priori sehr unwahrscheinlich, daß die Resorption schlecht oder unvollkommen ist. Auch die gleich noch zu besprechende Tatsache, daß eine Linksdrehung im Harn nur in den 2 ersten Stunden nach der Aufnahme feststellbar ist, spricht sehr dagegen.

Um die Ausscheidungsverhältnisse im Urin zu prüfen, bekamen gesunde Menschen nüchtern 250 g Kaffee mit 20 g Lävoglucosan, der Harn wurde dann in stündlichen Perioden auf reduzierende, vergärende und polarimetrische Wirkung untersucht.

Folgendes Beispiel an einem stoffwechselgesunden Kranken mit Lues cerebrospinalis möge das illustrieren:

Tabelle II.

Zeit 4. IX. 1923	Nahrungs-zufuhr	Harn-menge	reduzierende Fähigkeit des Urins	Ver-gärungsfähigkeit des Urins	polari-metrisch	g ausge-schiedenes Lävo-glucosan pro Std.	g Lävo-glucosan im ganzen
6—7h p. m.	nüchtern	88 ccm	0	0	0		
7—8h	250 g Kaffee mit 20 g Lävo-glucosan	128 „	0	0	— 0,8 %	= 0,803	
8—9h	keine Zufuhr	170 „	0	0	— 0,2 %	= 0,267	1,070
9—10h	keine Zufuhr	—?	0	0	0		

1) Zur Kenntnis d. Caramelwirkung (Versuche mit Lävoglucosan), Rostock 1923.

Veränderungen sind nur bei der polarimetrischen Untersuchung aufgetreten. Es kam in den beiden ersten Stunden der Aufnahme des Lävoglucosans zu einer Linksdrehung, die nur in der ersten Stunde stärker ausgesprochen war, ein Beweis, daß die Resorption des Präparates offenbar außerordentlich rasch vor sich geht. Berechnet man unter Zugrundelegung der spezifischen Drehung des Lävoglucosans die Menge der unverändert ausgeschiedenen Substanz, so ist die Menge mit 1,070 g (= ca. 5% des Ausgangsmaterials) als sehr klein anzusprechen. Daß es sich tatsächlich um Lävoglucosan handelt, läßt sich durch den Nachweis von reduzierender Glucose nach Kochen mit verdünnter Schwefelsäure leicht feststellen.

Von besonderem Interesse war natürlich die Wirkung des Lävoglucosans im Organismus des Diabetikers. Herr Professor Curschmann hatte die Freundlichkeit uns zu gestatten, derartige Versuche an poliklinisch eingewiesenen Kranken der inneren Klinik durchzuführen, wozu wir ihm zu großem Danke verpflichtet sind; andere Versuche wurden an klinisch genau überwachten Privatpatienten angestellt.

Der Ausfall aller Versuche war stets der gleiche. Niemals kam es zu einer Steigerung der Glycosurie, 5—10% des Lävoglucosans erschienen im Harn wieder, eine etwa bestehende Acidose wurde günstig beeinflußt.

Folgende Beispiele mögen das illustrieren.

Bei dem Kranken Fl. (Tabelle III, handelte es sich um einen mittelschweren Diabetiker, der auch nach mehren Gemüsefettagen nicht ganz zuckerfrei und acidosefrei zu bekommen war.

Verglichen wurden an zwei aufeinander folgenden Tagen in dreistündlichen Perioden Zucker- und Acetonkörperausfuhr mit und ohne eine Zulage von 30 g Lävoglucosan zu einer Grundkost von 100 g Spinat, 50 g Speck und 250 g Kaffee. Die titrimetrisch nach Benedict festgestellte Zuckerausscheidung ist in beiden Vergleichsperioden die gleiche, so daß eine Zuckerbildung aus Lävoglucosan ausgeschlossen ist. Sehr charakteristisch ist dagegen der Einfluß auf die Acidose, unter Lävoglucosanwirkung sinkt sie auf den zehnten Teil ab (von 0,48 auf 0,047 g).

Für die Lävoglucosanwirkung bei einem etwas leichteren Diabetiker mit allerdings sehr niedriger Toleranz bringt Tabelle 4 den charakteristischen Verlauf in 4 Stundenperioden.

Auch hier keine Spur von Zucker, dagegen deutliche Linksdrehung, die aber auch hier nur in der 1. Stunde mit 0,8% etwas

Tabelle III. Versuchsreihen an Fl, 20j, an den Vortagen Gemüsediät.

Datum	Zeiten	Nahrungzufuhr	Urinmenge	Zuckermenge, stdl. nach Benedict	pro Versuch Gesamtzucker	Zuckermenge polarimetrisch pro Stunde	Zuckermenge polarimetrisch pro Versuch	Aceton qualitativ	Essigsäure qualitativ	Gesamtacetonpro Versuch
26. VII. 1923	10—11ᵃ m.	um 9⁴⁵ 50 g Speck 100 g Spinat 250 g Kaffee	110 ccm	0,63 g		0,3%=0,33 g		+	+	
	11—12ᵃ 12—1ᵃ	—	60 ccm 220 ccm	0,435 g 0	1,065 g	0,3%=0,18 g 0	0,51 g	schw. +	+ +	0,48 g
27. VII. 1923	7—8ᵃ	um 6⁴⁵ 50 g Speck 100 g Spinat 250 g Kaffee +30 g Lävogluc.	90 ccm	0,315 g		0,2%=0,18 g		+	schw. +	
	8—9ᵃ 9—10ᵃ	—	100 ccm 75 ccm	0,425 g 0,335 g	1,075 g	0,2%=0,20 g 0,2%=0,15 g	0,53 g	schw. + schw. +	schw. + +	0,047 g

Tabelle IV. Versuchsreihe bei dem 36j. B.

Versuchsperiode Nr.	Zeit	Urinmenge ccm	Ausfall von Nylander's Probe	Ausfall der Acetonprobe	Polarimetrisches Verhalten	Ausgeschiedenes Lävoglucosan berechnet aus der Linksdrehung
1. Stunde (vor Lävoglucosan)	23. VII. 1923 9½—10ᵃ	80 ccm	negativ	negativ	0%	0%
2. Stunde (im direkten Anschluß an Darreichung von 26 g Lävoglucosan)	10—11ᵃ	110 ccm	"	"	—0,8%	=0,630 g
3. Stunde (2.Stde. nach Lävoglucosan)	11—12ᵃ	120 ccm	"	"	—0,2%	=0,188 g
4. Stunde (3.Stde. nach Lävoglucosan)	12—1ᵃ	130 ccm	"	"	0,0%	} =0,578 g

11*

Tabelle V. Versuchsreihe bei dem 32j. Bankbeamten W. Ru.

Datum 1923	Versuchs-abschnitt	Zusammensetzg. der Kost	Kohle-hydrat-gehalt	Urinmenge	Zucker titrimetrisch	Zucker gärungs-analytisch (Lohnstein)	Zucker polari-metrisch	Acetessig-säure-aktion	Gesamt-aceton	Besonderes
19. 20.X.	3. Behandlungstag 7¹⁵–9¹⁵	30 g Fett, 2 Eier, 30 g Käse, 600 g Gemüse (Spargel, Spinat), 1 l Bouillon = 12 Cal. pro kg	30 g	2700 ccm	30,8 g	—	—	++	4,74 g	die verwandte Hefe wurde nicht auf Selbstgärung untersucht. Vielleicht erklärt sich durch letztere der zu hohe Wert
20.–21.X.	4. Behandlungstag 7¹⁵–9¹⁵	250 ccm Kaffee	0	114 "	4,4 g =2,03 g pro Std.	4,5 g	4,3 g	++	0,19 g	
	9¹⁵–12¹⁰	250 g Tee mit 30 g Lävoglucosan (125 Cal.)	30 g	162 "	5,35 g =1,94 g pro Sdl.	6,48 g	2,9 g	++	0,3 g	
	12¹⁰–1¹⁰	—	—	48 "	1,40 g	1,49 g	1,4 g	+	0,1 g	
	1¹⁰–8¹⁰ am folgenden Morgen	30 g Fett, 2 Eier, 50 g Käse, 600 g Spargel u. Spinat, 1 l Bouillon	30 g	1000 "	13,0 g	24,0 g?	12,0 g	+	1,4 g	
	Gesamttag	vgl. vorher	60 g	1324 "	24,15 g	—	20,6 g	+	1,99 g	

größer ist und im ganzen auf L. berechnet nur 0,878 g beträgt, also noch nicht einmal 4½ % der aufgenommenen Menge.

Schließlich sei noch eine Beobachtungsreihe bei einem recht schweren Diabetiker (Tabelle V) mitgeteilt, der erst durch Insulin zucker- und acidosefrei zu bekommen war.

Am 3. Gemüsetag schied er noch 30,8 g Zucker und 4,74 g Aceton aus. Am folgenden Tage erhielt er zur bisherigen Kost eine Zulage von 30 g Lävoglucosan, das die Zuckerausscheidung sehr mäßig (auf 24,15 g), die Acidose dagegen sehr wesentlich auf unter die Hälfte (1,99 g) herabdrückte.

Dieser Tag wurde in einzelne Abschnitte zerlegt. Auch hier zeigte sich wieder, daß die auf die Stunde umgerechnete Zuckerausscheidung in den Stunden unmittelbar nach Lävoglucosan sogar eher etwas niedriger (1,94 g) als vorher im Nüchternzustand (2,03 g) ausfiel. Die günstige Wirkung auf die Acidose trat erst in den späteren Stunden deutlich zutage.

Daß der phorizinvergiftete Hund sich genau so verhält wie der Diabetiker, zeigt Tabelle VI, die wir Herrn Dr. Otto Martiensen verdanken.

Tabelle VI. Versuchsreihe an einem phlorizin-diabetischen Hunde.

Datum 1923	Nah-rungs-zufuhr	Ge-wicht kg	Urin-menge	g Zucker gärungs-analytisch	g Zucker (Benedict)	g N	$\frac{D}{N}$	Bemer-kungen
19.–20. VI.	Hunger Ringer per Sonde.	5,85	570 ccm	21,6	19,38	3,537	6,1	1 g Phlorizin in Öl
20.–21. VI.	"	5,80	804 "	16,9	14,48	2,564	6,6	
21.–22. VI.	"	5,60	400 "	16,0	10,40?	3,035	5,2	1 g Phlorizin in Öl
22.–23. VI.	"	5,42	920 "	17,48	23,02	4,278	4,1	
23.–24. VI.	+8 g Lävogluc.	5,25	600 "	17,35	18,6	1,994	8,7	1 g Phlorizin in Öl

Die Zuckerausscheidung bleibt gärungsanalytisch unverändert, nach Benedict nehmen die Zahlen sogar ab.

Besonders deutlich ist die Einwirkung auf den Eiweißumsatz, der auf unter die Hälfte des Vortages absinkt, auch hier ein völliges Analogon zur Wirkung des Caramels.

In einzelnen Fällen wurde auch die Wirkung auf den Blutzucker (nach Benedict) untersucht.

Wie die beiden Beispiele in Tabelle VII zeigen, kommt es nach 20 g Lävoglucosan nicht nur zu keiner Steigerung, sondern vielmehr zu einem deutlichen Absinken des Blutzuckers in den folgenden Stunden.

Tabelle VII.

Zeit	Blutzucker- zahlen bei B.	Blutzucker- zahlen bei Fl.	Blutzucker- zahlen bei Dr. G.
vor Lävoglucosan	0,135 %	0,18 %	0,218
¾ Std. nach 20 g Lävoglucosan	0,08 %	0,18 %	0,20
2 Std. nach Lävoglucosan	0,12 %	0,125 %	0,21
4 Std. nach Lävoglucosan	0,10 %	0,15 %	—

In diesem Punkte besteht ein prinzipieller Unterschied gegenüber dem Caramel, denn nach den Untersuchungen von Magin und Turban[1] resultiert dort, wenn auch nur vorübergehend und wenig ausgesprochen, ein Anstieg der Werte. Allerdings ist dabei zu bedenken, daß selbst die Mercksche Caramose noch bis zu 10 % reduzierende Substanz enthalten kann, während Lävoglucosan überhaupt nicht mehr reduziert.

Faßt man das bisher untersuchte Verhalten des Lävoglucosans im normalen und diabetischen Organismus zusammen, so läßt sich feststellen, daß die Fermente des Magendarmkanals dieses Zuckeranhydrid nicht zu spalten vermögen, daß ca 5—10 % im Harne wieder erscheinen, daß jede glycosurische Wirkung fehlt, der Blutzucker sogar etwas herabgesetzt wird, während die Acidose sehr günstig beeinflußt und die Eiweißverbrennung eingeschränkt wird.

In allen wesentlichen Punkten besteht also eine weitgehende Übereinstimmung in der biologischen Wirkung zwischen Caramel und Lävoglucosan, nur tritt im letzteren Falle die Wirkung des anhydrierten Zuckers noch klarer und reiner zutage, da wir hier ein reines Präparat vor uns haben.

Ganz analog wie das Lävoglucosan verhält sich nach einer schriftlichen Mitteilung von Kerb, der darüber an anderer Stelle berichten wird,[1] das α-Glucosan.

Nach diesen Untersuchungen kann es wohl keinem Zweifel unterliegen, daß die biologisch jetzt zum ersten Male näher untersuchten reinen Glucosane tatsächlich die

1) l. c.

Hauptträger der günstigen Wirkung der gerösteten Kohlehydrate im diabetischen Organismus sind. Den Mechanismus der Wirkung der Glucosane aufzuklären, ist die Aufgabe weiterer Untersuchungen.

Es bestehen anscheinend zwei Hauptmöglichkeiten. Entweder reiht sich das Lävoglucosan, vielleicht durch Wasseraufnahme irgendwie in den gewöhnlichen Kohlehydratstoffwechsel ein, möglicherweise indem es die Glycogenbildung befördert, wofür man das merkwürdige Verhalten des Blutzuckers anführen könnte, oder aber der komplizierte, allerdings sehr widerstandsfähige Ring wird oxydativ gesprengt und in einer noch ganz unbekannten, vielleicht von den gewöhnlichen Kohlehydraten abweichenden Art abgebaut.

So entstehen auf diesem neuen Gebiete der Kohlehydratphysiologie eine Menge interessanter Fragen. Durch die vorliegenden Untersuchungen ist zunächst eine sichere Basis geschaffen worden, auf der weiter aufgebaut werden kann.

Wegen der besonders günstigen Wirkung des Lävoglucosans beim Diabetes sowie seines süßlichen Geschmackes würde sich therapeutisch und diätetisch dies Zuckeranhydrid als Zuckerersatz sehr gut eignen, doch ist bei den hohen Herstellungskosten an eine derartige Verwendung vorläufig leider nicht zu denken.

The measurement and character of caramel colour

D. W. GROVER

Summary. The colour of commercial caramels has been studied by measuring the extinction of monochromatic light (λ 0·4 to 0·7 μ) by solutions in water over a concentration range of 0·02–2·5 g/100 ml. The Lambert–Beer 'law' is proved to apply within the accuracy of measurement, and a straight line relationship between log E and λ is established. Similar observations are made for beverages containing caramel.

Based on these measurements, the colour characteristics of the materials are derived in terms of the Comité Internationale de l'Eclairage (C.I.E.) system, providing explanations for the differences and similarities between caramels, the difficulty of visual matching, and the changes in colour which occur on dilution.

A proposal is made for the assessment of the colouring power of caramel by measurement of extinction at wavelengths of 0·5 and 0·6 μ.

Symbols

λ = Wavelength in microns.

I_0 = Intensity of incident light.

I = Intensity of transmitted light.

T_λ = Transmission = I/I_0, at wavelength λ.

E_λ = Extinction = $- \log T_\lambda$.

l = Length of light path (cm).

c = Concentration (g/100 ml).

Es_λ = Specific extinction at wavelength λ.

λd = Dominant wavelength.

P_e = Excitation purity (saturation).

F_0 = Luminous flux of incident light.

F = Luminous flux of transmitted light.

E_f = Luminous extinction = $- \log F/F_0$.

Introduction

This paper is concerned with the physics of caramel colour. The chemical and technological aspects of the use of caramel as a colouring material are not considered. There are four main topics: (1) the spectral extinctions of caramel solutions, (2) the Lambert–Beer 'law', (3) caramel colour in the C.I.E. system, and (4) assessment of colorial power.

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Materials

Commercial caramels used for this investigation are listed in Table 1.

TABLE 1. Caramels investigated

Code	Application and type
A	Acid beverages; negative
B	Brewing; positive
C	Not known
E	Brewing; positive
F	Brewing
H*	Brewing
I	Spirits; alcohol compatible

*Probably deteriorated by age.

Part 1. Spectral extinctions of caramel solutions

Methods

Aqueous solutions of caramel containing 2·5 g/100 ml.were prepared. In one instance where the solution was obviously turbid it was filtered clear. Solutions containing 0·5, 0·1 and 0·02 g caramel/100 ml were then made by successive dilution. Extinctions were measured from $\lambda = 0.38\ \mu$ to $\lambda = 0.70\ \mu$ with a Unicam 500 Spectrophotometer using 1-cm glass cells. In some instances readings were made at λ intervals of 0·01 μ or closer. However, after it was found that the log E *vs* λ plot is an almost straight line, readings were taken at wider intervals, e.g. 0·05 μ.

Results and discussion

Too many individual readings were taken for these to be conveniently tabulated. Instead they are shown graphically. The plots of $E\lambda$ against λ for all caramels tested are smooth curves without breaks, showing high extinctions at the blue end of the spectrum and low extinctions at the red end. This result is at variance with the observations of Truhaut *et al.* (1961) who reported maximum extinctions at $\lambda = 0.41$ μ.

It will be established in Part 2 (p. 315) that, for monochromatic light, the extinction of caramel solutions is proportional to the optical path length and concentration (the Lambert–Beer 'law'). For the moment the validity of this will be assumed. This is expressed in the equations:

$$E\lambda = Es\lambda \times lc,$$ (1)

$$\log Es\lambda = \log E\lambda - \log lc.$$ (2)

Plots of log Es_λ against λ for caramels A, B, C, E, F and I are shown in Fig. 1.* It is seen that they approximate very closely to straight lines. By the usual mathematical procedure the best fitting straight lines have been found and are defined below by regression coefficients of log Es_λ on λ in the general equation:

$$\log Es_\lambda = a + b\lambda. \tag{3}$$

Values of a and b for the various caramels are tabulated in Table 2.

TABLE 2. Regression coefficients of Es_λ on λ for commercial caramels in equation log $Es_\lambda = a + b\lambda$

Caramel	a	b
A	3·07	−5·08
B	3·36	−5·11
C	3·23	−5·12
E	3·20	−5·22
F	3·28	−5·21
H	3·06	−4·76
I	3·84	−6·56

Similar plots for a pale ale, an Australian whisky and a caramel-coloured cola drink are shown in Fig. 2. The regression coefficients for these beverages are given in Table 3.

These figures can be compared with those for North American beers, calculated from extinction data published in 1962 (Stone & Miller, 1962). The λ vs log Es_λ relationships when plotted give almost straight lines for which the regression coefficients are given below:

	'a'	'b'
Canada (7)*	0·25	−5·82
New York (29)	0·10	−5·68
Wisconsin (13)	0·30	−6·27
New Hampshire (5)	−0·01	−5·74
New York (6)	−0·08	−5·69
Wisconsin (9)	0·16	−6·35

* The numbers are those in the original paper.

* For convenience of plotting the graph shows log $Es_\lambda + 5\lambda$. This in no way affects the discussion.

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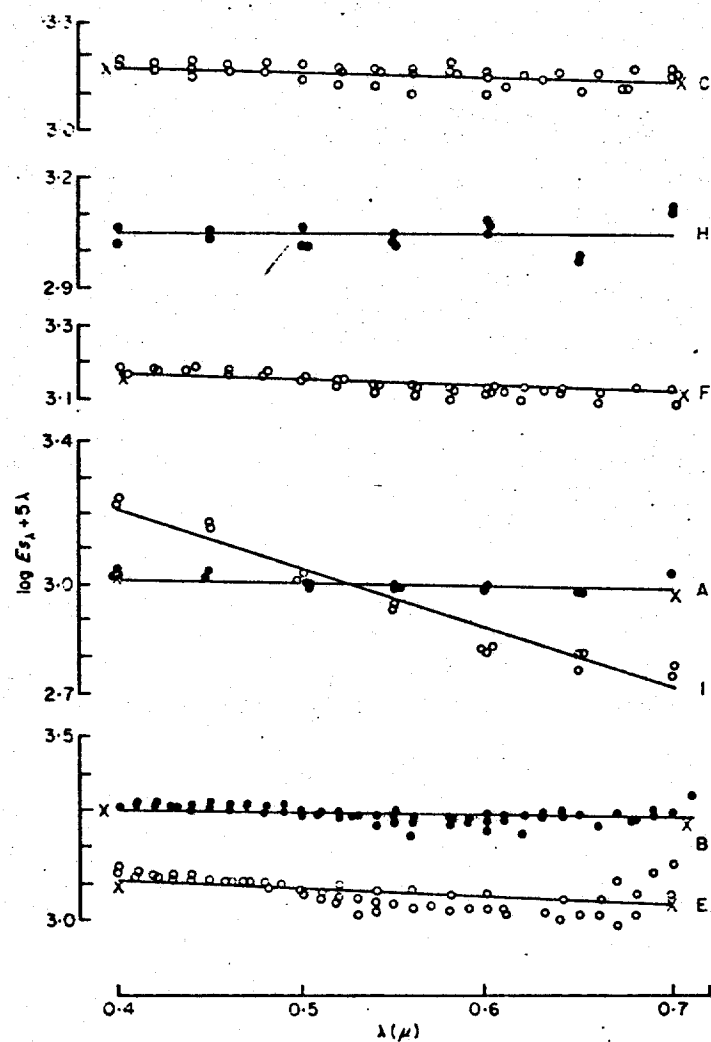
·Fig. 1. Relationship between wavelength and specific extinction for some caramels.

TABLE 3. Regression coefficients of log Es_λ on λ for beverages in equation log $Es_\lambda = a + b\lambda$

Beverage	a	b
Pale ale	0·10	·—5·57
Whisky	0·28	—5·76
Cola drink	0·37	—5·10
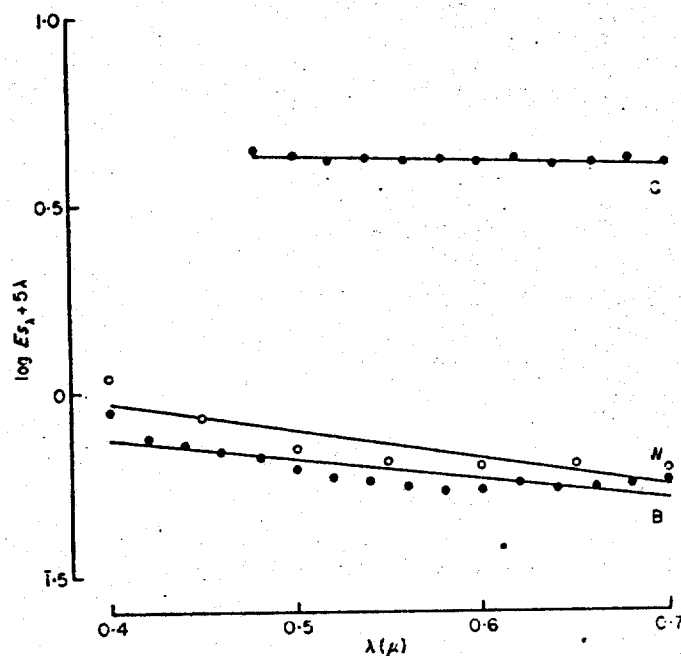
Fig. 2. Relationship between wavelength and specific extinction for some beverages. W, Whisky; B, beer; C, cola.

Discussion

The '*b*' coefficients for caramels A, B, C, E, F and the cola drink are almost equal, with a mean of −5·15. The lines of slope −5·15 which most closely correspond with experimental data fall between the points marked 'X' in Fig. 1. These are so close to the 'best' lines (as drawn in full) as to be almost indistinguishable. A consequence of identical values of '*b*' is that a solution of one of these samples can be precisely matched in spectral transmission by a solution of any of the others by suitably adjusting the concentration.

Part 2. The Lambert–Beer (LB) law

This 'law', contained in two propositions propounded respectively by Lambert and Beer, applies only to homogeneous monochromatic radiation. It has been expressed verbally in several ways. Mathematically it is contained in equations (1) and (2). The validity of the LB law has been established for sugar house products—which are similar in many ways to caramel (Stone & Miller, 1962).

One test of the LB law is to find out whether Es_λ changes as the concentration changes. It will be seen in Fig. 1 that for each caramel log Es_λ is almost independent of *c*. There is some scatter of the observations, notably at higher wavelengths where the use of a

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logarithmic function exaggerates the error in measuring small values of E. To establish more formally that $E_{5\lambda}$ is independent of c, the divergence between observed values of $E_{5\lambda}$ and the best fitting line have been correlated with c, for three wavelength ranges. The results are given in Table 4.

TABLE 4. Correlation between deviations from best fitting straight line and concentration

Wavelength range	No. of observation	Correlation coefficient	Probability	Regression coefficient log $E_{5\lambda}$ on c
0·4–0·49	52	0·223	0·1	—
0·5–0·59	70	0·269	0·023	0·021
0·6–0·71	75	0·209	0·1	—

Although there is a significant correlation in one range the regression coefficient of log $E_{5\lambda}$ on c is very small, a concentration change of 0·1 being associated with a change of only 0·5% in the specific extinction. It seems likely that the observed correlation is due to a small systematic observational error, rather than to any real departure from the LB law.

The validity of the LB law has also been tested visually in white light. A Hellige colorimeter was used for this purpose. Solutions of different concentrations of the caramel were placed in the two cells. The length l_1 was varied and the length l_2, required to obtain a match, was observed. Results are given in Table 5, where the observed values of l_2 are compared with values calculated on the basis of the LB law.

The small differences between observed and calculated values of l_2 are not systematic and can be assumed to be due to experimental error.

It is concluded that the LB law applies to solutions of commercial caramels within the limits of accuracy of the present experimental observations, and within the limits of practical importance for the use of caramels as colouring substances.

Part 3. Caramel colour in the C.I.E. system

The value of caramel lies in the character of light transmitted by the beverages and other foodstuffs in which it is used. This is obviously true for transparent materials viewed against the light. It is also true for coloured objects viewed by reflected light, when the coloured effect is produced by penetration of the light a small distance into the coloured material followed by internal reflection.

The visual colour depends on the distribution of intensity of the various radiations in the visual range. Although this can readily be measured with a spectrophotometer, interpretation in terms of visual effect is a complex and incompletely understood problem. In order to bring the relationship of distribution of radiation and visual colour into a form amenable to mathematical treatment, certain assumptions and conventions have to be accepted. The C.I.E. system of colour definition contains such

TABLE 5. The validity of the Lambert–Beer law with white light

| Left-hand cell | | | Right hand cell | | |
| | | | l_2 (cm) | | |
c_1 (g 100 ml)	l_1 (cm)	c_2 (g/100 ml)	Observed	Calculated	Difference
0·5	0·5	0·1	2·49	2·50	−0·01
		0·2	1·21	1·25	−0·04
		0·3	0·81	0·83	−0·02
		0·4	0·61	0·62	−0·01
		0·5	0·50	0·50	0·00
0·5	1·0	0·1	4·98	5·00	−0·02
		0·2	2·51	2·50	+0·01
		0·3	1·70	1·67	+0·03
		0·4	1·26	1·25	+0·01
		0·5	1·00	1·00	0·00
1·0	0·5	0·5	0·98	1·00	−0·02
		0·6	0·83	0·83	0·00
		0·8	0·62	0·62	0·00
		1·0	0·50	0·50	0·00
1·0	1·0	0·5	2·02	2·00	+0·02
		0·6	1·67	1·67	0·00
		0·8	1·29	1·25	+0·04
		1·0	1·10	1·00	+0·01

assumptions and conventions, but is well accepted as a good means of practical colou definition. In the C.I.E. system any mixed visible radiation is represented as a mixtu of three specific monochromatic radiations (e.g. $\lambda_1 = 0·700$ μ, $\lambda_2 = 0·546$ μ, λ_3 0·458 μ). The respective amounts of these required to give a match, called tl tristimulus (X,Y,Z) values of the mixed radiation, can be computed from the spectr transmission. Any mixed radiation can also be defined as a combination of whi light and monochromatic light of wavelength λd, where λd is the dominant waveleng of the mixed radiation. The colour and intensity of the mixed radiation are th completely defined by three parameters:

F = Luminous flux derived from the tristimulus values.

λd = Dominant wavelength.

P_e = Excitation purity.

The light transmitting properties of a transparent coloured medium can similar be defined by three characteristics, each equivalent to, or identical to one of the thr

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characteristics of the transmitted light discussed in the previous paragraph, and each independent of the intensity of the incident light.

These are:

E_f = Luminous extinction = $-\log F/F_0$ (for an equal energy source).

λd = Dominant wavelength.

P_e = Excitation purity.

The relationships between E_f and, respectively, λd and P_e for two hypothetical caramels are shown in Figs. 3 and 4. The caramels, marked respectively '5·15' and '6·56' are assumed to have the following $\log E\lambda$ equations:

$$\text{'5·15'} \quad \log E\lambda = 3·09 - 5·15\lambda + \log lc,$$
$$\text{'6·56'} \quad \log E\lambda = 3·84 - 5·56\lambda + \log lc.$$

In the figure are also marked the values of P_e and λd for the three beverages included in Table 3.

Discussion

The fact that the majority of caramels have parallel straight lines for the $\log Es\lambda$ *vs* λ plots (Fig. 1) means that any solution of one can be identically matched by a solution of the correct concentration of any of the others. Some materials, e.g. caramel I, covered by this paper are not alike in this way. Differences between them are illustrated in the graphs of Figs. 3, 4 and 5.

The dominant wavelength, λd, is of immediate interest. Fig. 3 demonstrates a familiar phenomenon—the change in colour from deep red to yellow which occurs when a caramel solution is progressively diluted. The dominant wavelength of light transmitted through dilute solutions of low extinction is about 0·575 μ, while for concentrated solutions of high extinction it is about 0·658 μ. The cola drink in a depth of 10 cm has $\lambda d = 0·619$ μ. As an indication of the visual quality of these wavelengths the colour ranges are set out in Table 6.

TABLE 6. Visual appearance of monochromatic light of various wavelengths

Colour ranges = 0·57–0·62 μ (Kelly, 1943)

Above 0·618	Red
0·596–0·618	Reddish orange
0·586–0·597	Orange
0·580–0·586	Yellowish orange
0·575–0·580	Yellow
0·570–0·575	Greenish yellow
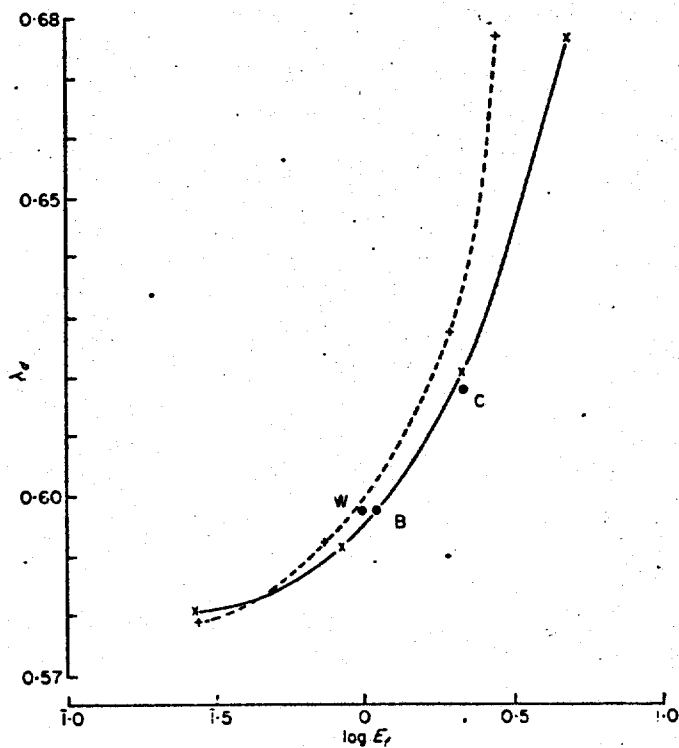
FIG. 3. Relationship of dominant wavelength to luminous extinction. $+$, Caramel '6·56'; $\times$, caramel '5·15'; W, whisky; B, beer; C, cola beverage.
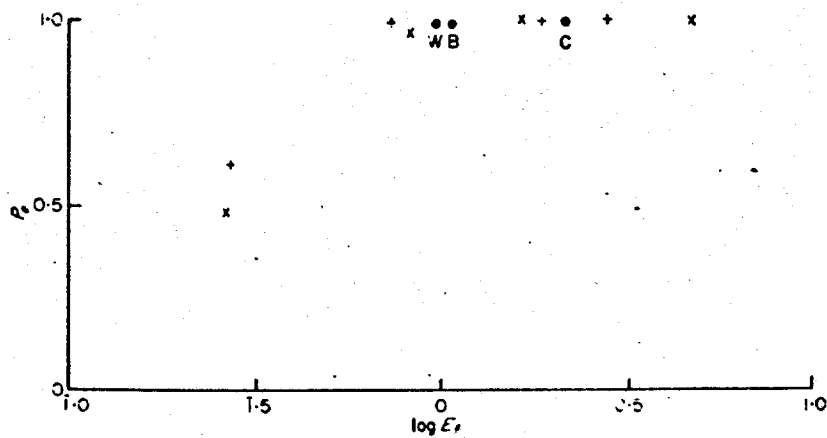
FIG. 4. Relationship of excitation purity to luminous extinction. $+$, Caramel '6·56'; $\times$, caramel '5·15'; W, whisky; B, beer; C, cola beverage.

Changes in saturation or extinction purity (P_e) with increase of concentration are shown in Fig. 4. Dilute solutions have a relatively unsaturated colour. More concentrated solutions have higher P_e values. When $E_f > 0.9$ (λd about 0·59), $P_e = 1$. Fig. 4 reveals differences between caramels '5·15' and '6·56'. The latter has greater P_e values when E_f is less than 0.

The LB law provides that $E\lambda$ is proportional to both the light path and the concentration, and it has already been established that this relationship holds for caramel solutions. However the luminous extinction E_f of caramel solutions is not proportional to $l \times c$. The luminous extinction increases less as concentration is increased than proportionality requires (see Fig. 5).

Part 4. Assessment of tinctorial power of caramels

Several methods of assessing caramel colour are in current use, and the caramel manufacturer is frequently required to specify tinctorial power in different ways for different buyers. The commonly employed methods are of three types:

(a) Visual comparison with some standard reference such as Lovibond (Salamon & Goldie, 1900) or European Brewing Convention (1950, 1952, 1953) glasses.

(b) Comparison with some standard (Truhaut, 1961) reference by means of an absorptiometer.

(c) Measurement of the extinction coefficient of a solution in monochromatic light at one or more wavelengths by means of a spectrophotometer (Gillette & Heath, 1954; Deitz, 1956).

The limitations of visual comparisons are well known. Nevertheless, for routine testing of caramel for a specific purpose reasonably consistent results are obtained Lovibond glasses have been in use for over 65 years. The E.B.C. comparator has been introduced more recently and is widely applied to caramels for the brewing trade.

Instrumental techniques give more definite and objective, but not necessarily more useful, information. From what has been learned about the light transmitting properties of caramel solutions it is obvious that a single measurement cannot provide a complete colour assessment.

Spectrophotometric measurement

Various suggestions have been made for the best wavelength for measuring the light extinction of caramel and caramel-like solutions (Gillette & Heath, 1954; Deitz, 1956). The International Commission for Uniform Methods of Sugar Analysis (1958) has proposed 0·42, 0·56 and 0·72 μ; 0·42 μ for lightly coloured material, 0·56 μ for dark materials and 0·72 μ as a basis for correcting for turbidity. In the absence of a spectrophotometer similar readings can be obtained on an absorptiometer using Ilford filters having appropriate transmission bands. The transmittance results however are not exactly equal to those obtained with a spectrophotometer.
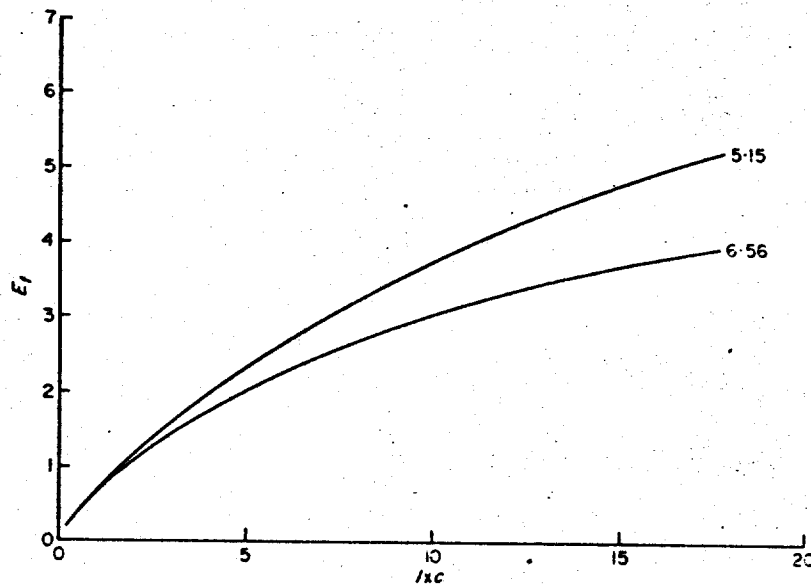
Fɪ. 5. Relationship of luminous extinction to optical path (*l*) by concentration (*c*) for two caramels.

Some theoretical basis for selecting the wavelength is provided as follows:

(a) If two caramels have the same values of '*b*' in the equation (3), their relativ colouring power will be the same whatever wavelength light is used for the com parison. If the '*b*' values are different, their relative colouring powers will depen on the wavelength of the light selected.

(b) As an index of the colouring power of a substance it is reasonable to use th extinction of light of a wavelength which is significant in determining the colou of the transmitted light. This criterion can be applied in selecting a wavelength c light for extinction measurement. The light which contributes the most to the luminou flux of the transmitted light is that for which $T_\lambda V_\lambda$ is maximum (where V_λ is th luminous efficiency of the light of wavelength λ).* This has been calculated for solution of caramel '5·15' for values of $lc = 0.5$ and 2.0. The maximum values of $T_\lambda V_\lambda$ for thes solutions occur at $\lambda = 0.60$ and $\lambda = 0.63$, respectively. A proportional change i transmission of light of these wavelengths therefore has a greater effect on the qualit of the transmitted light than the same proportional change in the transmission of ligh of any other wavelength.

* V_λ is the relative spectral luminous efficiency of monochromatic radiation of wavelength λ fo photopic vision as standardized in 1924 by the C.I.E. Consider two radiations of equal luminous flux and wavelengths λ and 0.555μ (the wavelength of maximum luminous efficiency, and relative energie respectively ε_λ and $\varepsilon_{0.555}$. Then $V_\lambda = \varepsilon_{0.555}/\varepsilon_\lambda$.

(c) The light absorbing properties of any of the caramels included in this stud are completely defined by the two parameters 'a' and 'b' in the equation 3. But 'a' and 'b' can be evaluated following the measurement of $E\lambda$ at two different wav lengths. The second wavelength is selected to be: (i) sufficiently close to 0·6 μ for g measurements of $E\lambda$ to be made without changing the concentration of the caram solution, and (ii) sufficiently far from 0·6 μ to provide an accurate base for calculatio A wavelength of 0·5 μ satisfies these conditions.

Proposed spectrometric procedure

It is proposed that the specific extinction Es_λ be measured at wavelengths 0·6 an 0·5 μ, the values so obtained to be designated, respectively Es (0·6) and Es (0·5). The colouring power of the caramel is then defined by two figures:
(i) Specific extinction (λ = 0·6) i.e. Es (0·6).
(ii) The value of b in the equation log Es_λ = $a + b\lambda$.
    This is derived from the equation:
    $b = -10 [\log Es (0·5) - \log Es (0·6)]$.

Es (0·6) gives a primary measure of the colouring power while b gives an indicatio of the change in the colouring power on dilution. Es (0·6) and 'b' figures of some of th commercial caramels included in Table 1 are listed below in Table 7.

TABLE 7. Tinctorial power of commercial caramels

	Es (0·6)	b
A	1·05	−5·08
B	1·97	−5·11
C	1·44	−5·12
E	1·17	−5·22
F	1·43	−5·21
I	0·80	−6·56

These figures are calculated from the data presented in Fig. 1, namely the be straight line through the plot of log Es_λ against λ.

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Industrial Bio-Test Laboratories, Inc.

Subacute Oral Toxicity of
Caramel Colorings in Dogs and Rats

Unpublished (1962)

NOTE

In this report caramels used are identified as follows:

1. Sample A, single strength
    Corn Products Refining Co., closed kettle
    Carbohydrate: starch hydrolysates
    Catalyst: ammonium hydroxide, sulfur dioxide
            and sodium bisulfite.
    Analysis: 0.6% N, 1.0% S

2. Sample B, double strength
    Corn Products Refining Co., closed kettle
    Carbohydrate: starch hydrolysate
    Catalyst: ammonium hydroxide, sulfur dioxide
            and sodium bisulfite
    Analysis: 1.4% N, 2.7% S

Industrial BIO-TEST *Laboratories, Inc.*
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS
Telephone CRestwood 2-3333

REPORT TO

CORN PRODUCTS REFINING COMPANY

SUBACUTE ORAL TOXICITY OF

CARAMEL COLORING (CODED SAMPLE A) - DOGS

Dogs 1/22/62

single strength acid-fast

1962

Table of Contents

REPORT TO

CORN PRODUCTS REFINING COMPANY

SUBACUTE ORAL TOXICITY OF

CARAMEL COLORING (CODED SAMPLE A) - DOGS

I. Introduction and Outline of Investigation

A sample of Caramel Coloring coded Sample A, was received from Corn Products Refining Company for the purpose of conducting a 90-day subacute oral toxicity study in beagle dogs.

The test material, Sample A, was incorporated into a basic diet at three graded levels and fed to three selected groups of six adult beagle hounds each, evenly divided as to sex, for a period of 90 days. The three dietary feeding levels corresponded to 6.0, 12.5 and 25 per cent of Sample A by weight respectively.

In addition to the three dose groups, a control group of six dogs was fed the basic diet containing no added test material.

The dogs in all groups were housed in kennels during the investigation, three dogs of the same sex and dose group being accommodated in a kennel.

At regular intervals during the investigation period observations

were made with respect to growth, food consumption, incidence of mortality, and reactions displayed. Periodic tests of liver and kidney function were also made plus complete studies of both blood and urine.

At the conclusion of the test, all dogs were sacrificed and gross and microscopic pathologic studies were conducted.

II. Investigational Procedure

A. Experimental Animals

The 24 dogs employed in the 90-day study were pure bred adult beagle hounds. The beagles were all eligible for A.K.C. registration and had been previously immunized against rabies, distemper, infectious hepatitis, and leptospirosis.

All dogs were under the observation of a staff veterinarian for two weeks prior to the start of the investigation during which time they were re-immunized.

B. Organization of Control and Test Groups

The organization of the test and control groups is shown in Table I.

C. Diets, Feeding and Food Consumption

The composition of the diets is presented in Table II.

TABLE I

Organization of the Test

Group	Dietary Level* Per Cent, w/w	Number of Test Animals Males	Females
Control	-	3	3
I	6.0	3	3
II	12.5	3	3
III	25.0	3	3

* The test diets were fed five days per week; control diet was offered to all dogs the remaining two days per week.

TABLE II

Dietary Constituents

Constituent	Control Group	Group I	Group II	Group III
		Per Cent Composition of Diets		
Dog Chow [a]	88.31	82.31	75.81	63.31
Beef [b]	10.05	10.05	10.05	10.05
Corn Oil [c]	1.64	1.64	1.64	1.64
Sample A	-	6.00	12.50	25.00
TOTAL	100.00	100.00	100.00	100.00

a. Pulverized Purina Dog Chow - Ralston Purina Company, St. Louis, Missouri
b. Ken-L-Burger - The Quaker Oats Company, Chicago, Illinois
c. Mazola Corn Oil - Best Foods, Division of Corn Products Co., New York, New York

Industrial BIO-TEST Laboratories, Inc.
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS
Telephone CRestwood 2-3030

February 15, 1962

FEB 19 1962

Dr. Dorothy Rathmann
Corn Products Company
P. O. Box 345
Argo, Illinois

Dear Dr. Rathmann:

This letter is in answer to your question regarding the report recently
sent to Corn Products entitled "Subacute Oral Toxicity of Caramel
Coloring (Coded Sample A) - Dogs".

On page 6 of the report it is indicated that the test ration was fed 5 days
per week and that, on each occasion, food consumption was recorded
after permitting the dogs one hour to consume the daily ration. This is
correct. On the remaining two days of each week (week-ends) the dogs
were offered equivalent amounts of control food and consumption of this
food was not recorded quantitatively. Food consumption data presented
in the report is applicable to test food only.

Very truly yours,

John H. Kay, Ph. D.
Associate Director

JHK:gm

All diets were prepared in the central diet room of the laboratories. The basic constituent from which all diets were constructed was pulverized Purina Dog Chow.

Following the appropriate additions of the constituents of any given diet, the mixture was thoroughly blended with a Hobart mixer. Diets were prepared weekly and stored in sealed metal cans at refrigerator temperature (4°C). One such can served to hold one day's ration for the dogs in any particular group.

Each morning, five days a week, the daily ration for each group (2220 grams/group = 370 grams/dog) was removed from the refrigerator and mixed with a precalculated quantity of water. The highest degree of palatability was obtained when the amount of water added approached two-thirds the weight of the dry food. After thorough blending, the dry food-water mix for each group was compressed into a large, circular, cake like mass and, with the aid of a specially constructed device resembling a large cookie cutter, it was divided into six equal portions for distribution.

All uneaten food was collected one hour after offering, returned to the diet room, weighed, and the mean food consumption for each sex was calculated and recorded.

D. Body Weights and Weight Gains

Initially, on the morning of the first test day, the body weight of each dog in every group was determined and recorded. Thereafter,

individual weighings were made weekly and the data recorded as an index to growth.

E. Mortality and Reactions

Checks for mortality and abnormal behavioral reactions were made daily during the investigational period.

F. Hematologic Studies and Urine Analyses

Blood studies, including determinations of hemoglobin concentration, erythrocyte count, and both total and differential leukocyte counts, were made at the beginning and at 45, and 90 days following the inception of the test. Urine analyses for reducing substances, albumin, and microscopic elements were also done at these time intervals.

G. Liver and Kidney Function Tests

Liver and kidney function tests, employing the sulfobromophthalein and phenolsulfonphthalein methods respectively, were conducted at the beginning and at 45 and 90 days following the inception of the test.

H. Gross and Microscopic Pathologic Studies

At the conclusion of the study, all surviving dogs in each group were sacrificed and subjected to complete gross and microscopic pathologic examination. The tissues and organs examined were: heart, aorta, trachea, lungs, liver, gall bladder, pancreas, esophagus, stomach, small intestine, large intestine, spleen, lymph nodes, kidneys, urinary bladder, gonads, prostate (males), uterus (females), pituitary, adrenal glands, salivary glands, thyroid, parathyroids, skeletal

muscle, bone, bone marrow, and representative portions of the central

and peripheral nervous systems.

III. Results

A. Body Weights

The weekly group mean and sex mean body weight data are presented in Tables III to V and illustrated graphically in Figures 1 to 3.

Mean weight gains over the 90-day test period, tabulated by sex and group, are presented in Table VI.

Examination of the weight data disclosed no evidence of adverse growth effects among test dogs. All test dogs gained weight essentially comparable to controls.

TABLE III

Mean Group Body Weights

Group	Mean Body Weights (kilograms) Days:													
	0	7	14	21	28	35	42	49	56	63	70	77	84	90
Control	5.0	5.1	5.3	5.4	5.4	5.7	5.8	5.8	5.9	6.1	6.4	6.4	6.4	6.5
Test Group I	5.7	6.0	6.0	6.1	6.2	6.4	6.6	6.5	6.6	6.6	6.8	7.0	7.0	7.0
Test Group II	7.0	7.2	7.2	7.4	7.4	7.6	7.7	7.8	7.9	8.0	8.2	8.4	8.6	8.6
Test Group III	6.3	6.4	6.6	6.7	6.8	7.0	7.0	7.2	7.4	7.5	7.6	7.8	7.8	7.8

TABLE IV

Mean Male Body Weights

Group	Mean Body Weights (kilograms) Days:													
	0	7	14	21	28	35	42	49	56	63	70	77	84	90
Control	4.5	4.4	4.5	4.7	4.7	5.1	5.3	5.5	5.6	5.9	6.2	6.2	6.2	6.2
Test Group I	6.0	6.3	6.2	6.4	6.4	6.5	6.7	6.7	6.8	6.9	7.1	7.2	7.3	7.3
Test Group II	8.1	8.3	8.5	8.8	8.8	9.0	9.2	9.4	9.5	9.4	9.7	10.1	10.3	10.6
Test Group III	6.3	6.5	6.6	6.9	7.0	7.1	7.3	7.5	7.7	7.7	7.9	8.0	8.0	8.1

TABLE V

Mean Female Body Weights

| Group | Mean Body Weights (kilograms) Days: | | | | | | | | | | | | | |
	0	7	14	21	28	35	42	49	56	63	70	77	84	90
Control	5.6	5.8	6.1	6.1	6.1	6.3	6.2	6.2	6.2	6.3	6.5	6.6	6.7	6.8
Test Group I	5.4	5.7	5.9	5.8	6.0	6.3	6.4	6.3	6.3	6.4	6.6	6.7	6.7	6.8
Test Group II	5.8	6.0	5.9	5.9	6.0	6.2	6.2	6.2	6.3	6.5	6.6	6.7	6.8	6.7
Test Group III	6.3	6.4	6.5	6.5	6.6	6.8	6.8	7.0	7.2	7.3	7.4	7.5	7.6	7.6

Figure 5
Mean Group Body Weight

Control Group
Group I - 6.0%
Group II - 12.5%
Group III - 25.0%

Figure 2
Mean Male Body Weights

Control Group
Group I - 6.0%
Group II - 12.5%
Group III - 25.0%
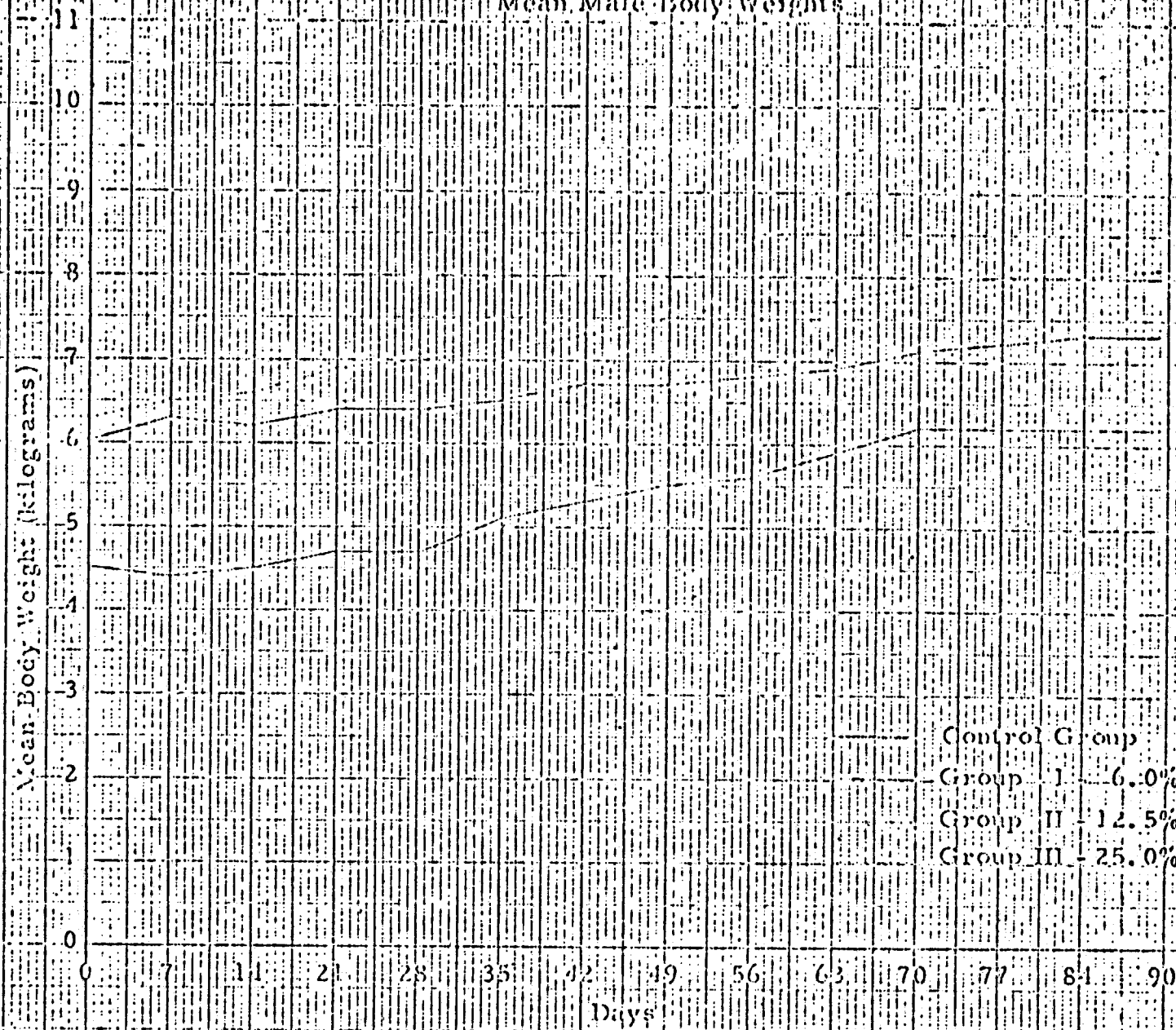
Figure 3

Mean Female Body Weights

Mean Body Weight (Kilograms)

Days

Control Group
Group I - 6.0%
Group II - 12.5%
Group III - 25.0%

TABLE VI

Mean Weight Gains

Group	Dietary Level Per Cent, w/w	Total Mean Weight Gains in 90 Days (kilograms)		
		Male	Female	Group Mean
Control	-	1.7	1.2	1.5
I	6.0	1.3	1.4	1.3
II	12.5	2.5	0.9	1.6
III	25.0	1.8	1.3	1.5

B. Food Consumption

The weekly group mean and sex mean food consumption data are presented in Tables VII to IX, and illustrated graphically in Figures 4 to 6.

All of the test dogs exhibited food consumption comparable to or slightly greater than control dogs.

Mean Group Food Consumption

Group	Mean Food Consumption (kg/dog/week) Days:												
	7	14	21	28	35	42	49	56	63	70	77	84	90
Control	1.06	1.36	1.58	1.35	1.46	1.86	1.52	2.04	1.87	2.03	1.92	2.43	2.10
I	1.27	1.38	1.66	1.48	1.12	1.48	1.43	1.91	1.86	1.80	1.74	2.05	2.02
II	1.75	1.90	1.92	1.96	2.06	2.09	2.03	1.98	2.23	2.09	2.12	2.38	2.50
III	1.62	1.75	2.00	1.72	1.91	2.13	2.14	2.22	2.38	2.20	2.26	2.56	2.61

TABLE VIII

Mean Male Food Consumption

Group	Mean Food Consumption (kg/dog/week) Days:
	7	14	21	28	35	42	49	56	63	70	77	84	90
Control	1.22	1.24	1.35	1.12	1.46	1.80	1.46	1.92	1.82	2.03	1.80	2.60	2.45
I	0.94	1.38	1.77	1.20	1.21	1.54	1.54	2.62	2.06	2.11	1.83	2.39	2.50
II	1.50	2.00	2.54	2.40	2.54	2.60	2.71	2.60	2.60	2.60	2.60	2.74	2.85
III	1.17	1.52	2.09	1.69	1.80	2.03	2.03	2.25	2.60	2.32	2.38	2.98	3.02

TABLE IX

Mean Female Food Consumption

Group	7	14	21	28	35	42	49	56	63	70	77	84	90
Control	0.91	1.47	1.80	1.58	1.46	1.92	1.58	2.15	1.92	2.03	2.03	2.26	1.74
I	2.00	1.38	1.54	1.77	1.03	1.43	1.32	1.20	1.66	1.48	1.60	1.71	1.54
II	2.00	1.80	1.30	1.52	1.58	1.58	1.35	1.35	1.86	1.58	1.64	2.03	2.15
III	2.08	1.93	1.92	1.75	2.02	2.34	2.26	2.20	2.15	2.08	2.14	2.14	2.20

Mean Food Consumption (kg/dog/week) — Days

Figure 1
Mean Group Food Consumption
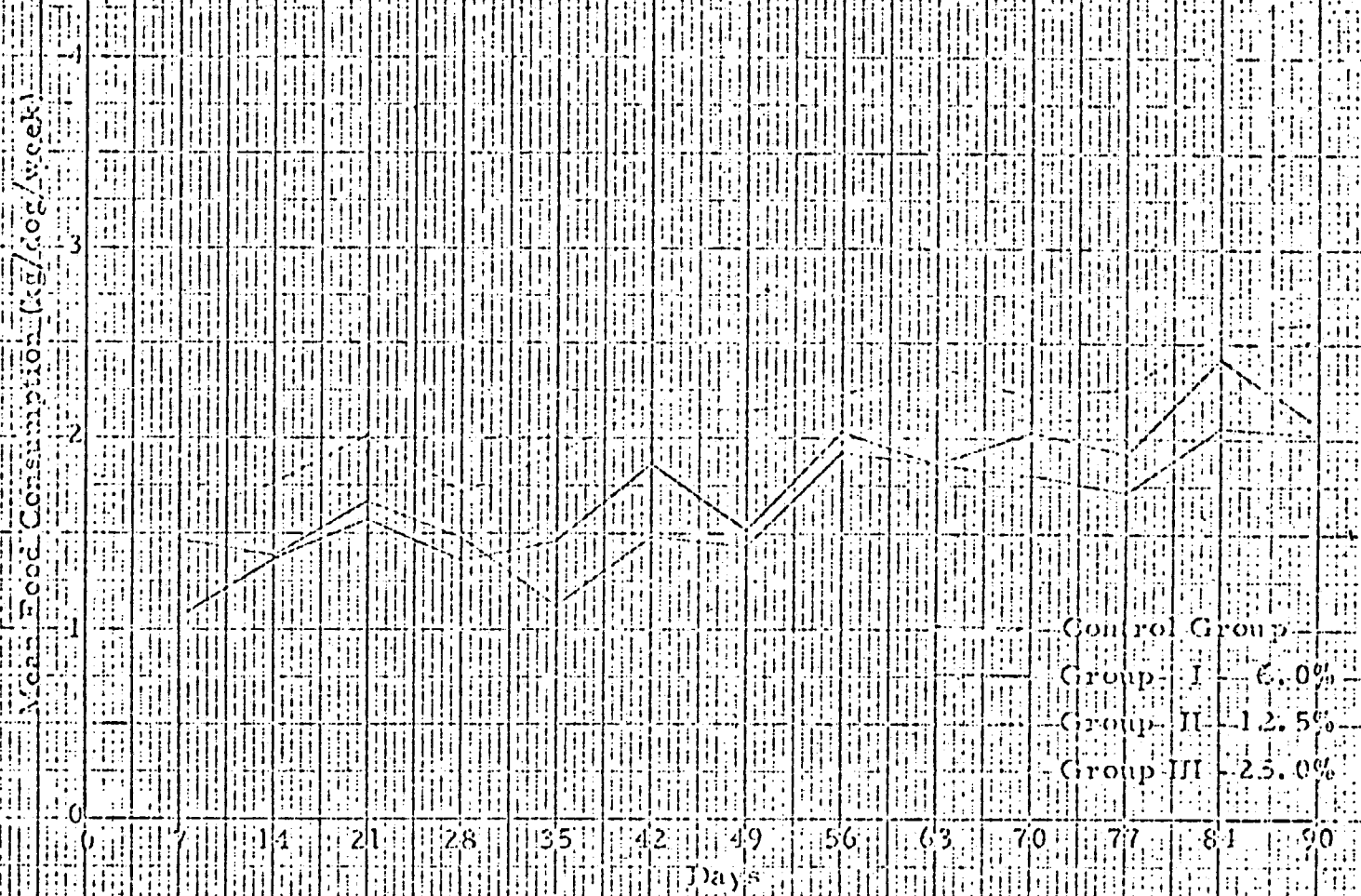
Figure 5

Mean Male Food Consumption

Control Group
Group I - 6.0%
Group II - 12.5%
Group III - 25.0%

Days

Mean Food Consumption (gr./dog/week)
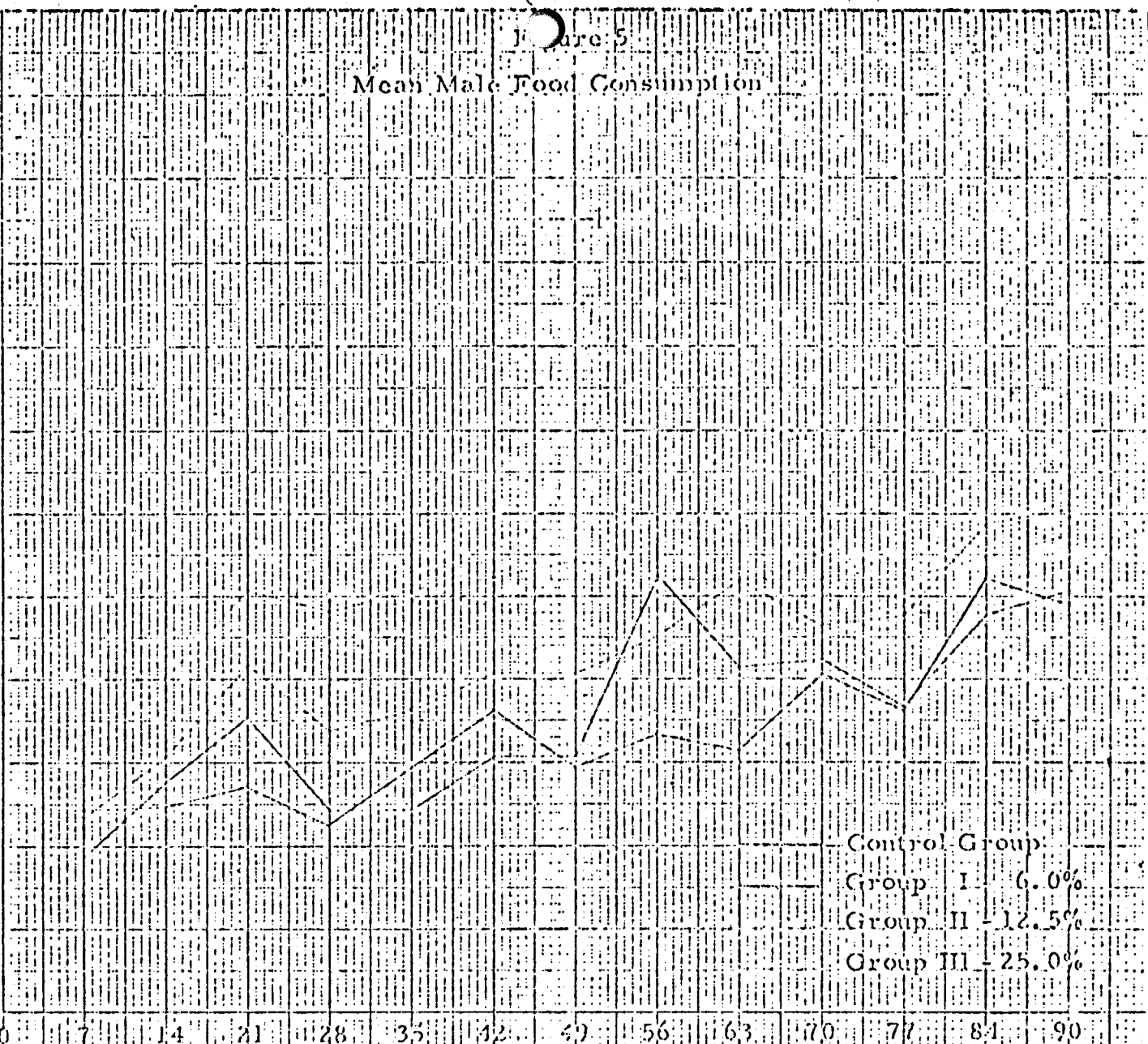
Figure 6

Mean Female Food Consumption

Control Group
Group I – 6.0%
Group II – 12.5%
Group III – 25.0%

Days

Mean Food Consumption (kg/dog/week)
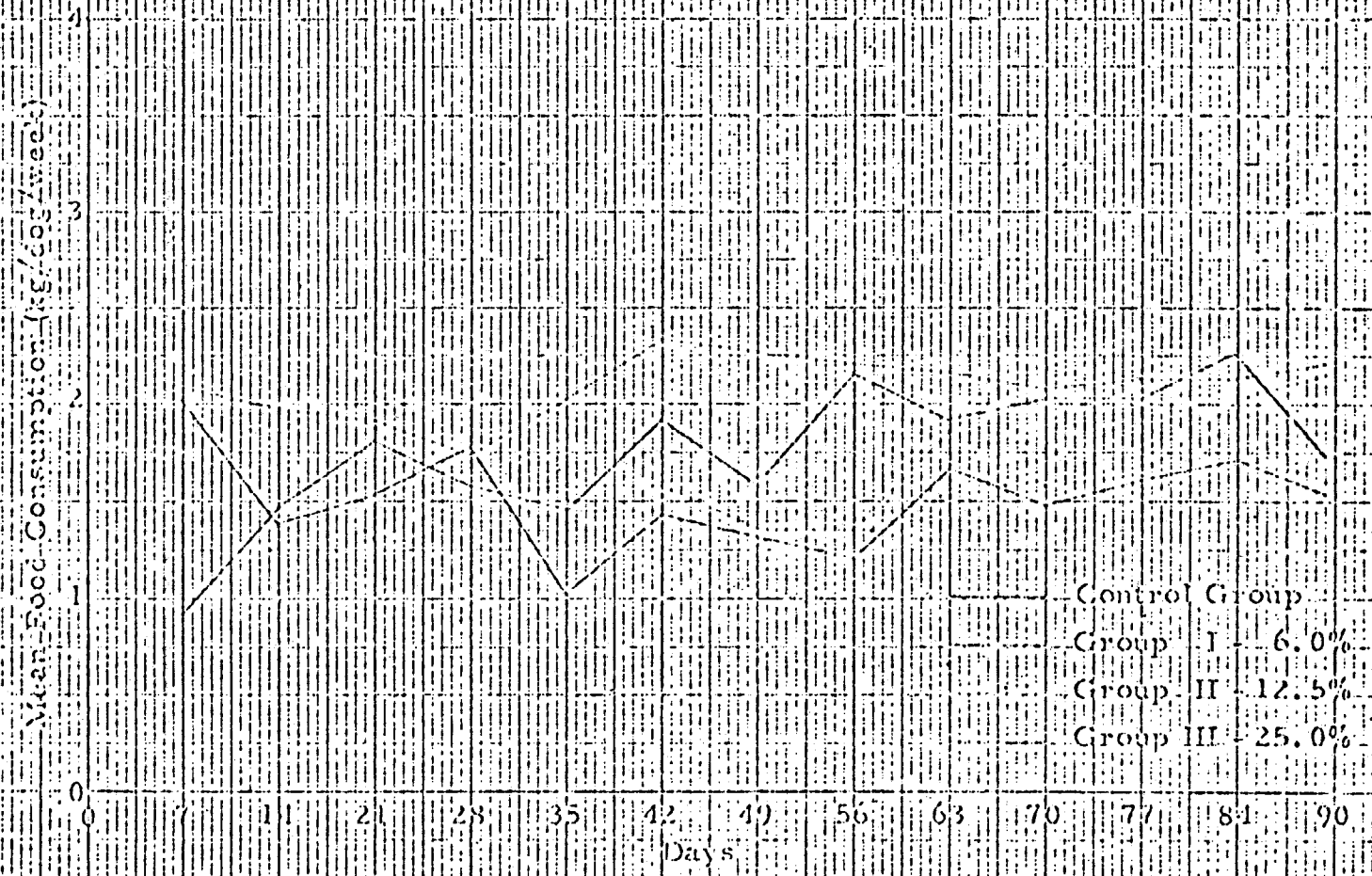
C. <u>Mortality and Reactions</u>

There were no mortalities or untoward reactions noted during the 90-day test period.

D. Hematologic Studies

The results of the hematologic studies conducted upon individual dogs in all groups are presented in Tables X through XVII.

No significant abnormalities were noted.

Dog Number and Sex	Hemoglobin, g/100 ml Days:			Erythrocytes, Millions/mm³ Days:			Leukocytes, Thousands/mm³ Days:		
	0	45	90	0	45	90	0	45	90
1-M	10.2	11.9	10.8	5.2	6.4	5.5	12.9	15.3	17.0
2-M	12.6	13.6	12.7	5.6	5.3	6.6	15.9	14.3	15.8
3-M	13.3	13.7	12.1	6.3	6.2	5.9	14.8	13.9	14.3
Male Mean	12.0	13.1	11.9	5.7	6.0	6.0	14.5	14.5	16.0
4-F	11.5	11.9	14.5	5.5	6.2	7.3	23.4	14.2	12.5
5-F	13.3	12.2	14.1	5.4	6.8	6.8	19.3	14.7	14.6
6-F	13.7	12.3	13.7	6.8	5.9	7.1	14.7	15.2	13.9
Female Mean	12.8	12.1	14.1	5.9	6.3	7.1	19.1	14.7	13.7
Group Mean	12.4	12.6	13.0	5.8	6.2	6.6	16.8	14.6	14.8

TABLE XI

Hemoglobin, Erythrocyte, and Total Leukocyte Count

Test Group I

Dog Number and Sex	Hemoglobin, g/100 ml Days:			Erythrocytes, Millions/mm^3 Days:			Leukocytes, Thousands/mm^3 Days:		
	0	45	90	0	45	90	0	45	90
1-M	10.4	12.2	10.7	5.7	6.6	5.9	22.4	15.8	23.9
2-M	10.3	13.4	12.2	5.3	5.8	6.2	23.8	14.7	17.6
3-M	11.7	13.4	12.6	5.5	5.7	6.3	19.6	16.3	20.1
Male Mean	10.8	13.0	11.8	5.5	6.0	6.1	21.9	15.6	20.5
4-F	11.7	13.2	12.5	6.1	6.8	6.9	20.7	19.3	14.9
5-F	11.4	12.9	13.5	5.5	5.8	6.0	25.4	18.3	14.8
6-F	12.3	13.3	12.6	6.3	5.5	6.8	22.1	17.6	15.9
Female Mean	11.8	13.1	12.9	6.0	6.0	6.6	22.7	18.4	15.2
Group Mean	11.3	13.0	12.4	5.8	6.0	6.4	22.3	17.0	17.6

TABLE XII

Hemoglobin, Erythrocyte, and Total Leukocyte Count

Test Group II

Dog Number and Sex	Hemoglobin, g/100 ml Days:			Erythrocytes, Millions/mm^3 Days:			Leukocytes, Thousands/mm^3 Days:		
	0	45	90	0	45	90	0	45	90
1-M	11.5	12.9	13.2	5.6	5.8	6.5	21.7	18.3	20.4
2-M	11.3	13.6	12.1	5.2	5.9	6.2	40.0	26.3	25.7
3-M	12.7	13.7	13.8	5.7	6.2	6.8	19.3	20.1	21.3
Male Mean	11.8	13.4	13.0	5.5	6.0	6.5	27.0	21.6	22.5
4-F	12.7	14.1	11.4	6.1	5.6	5.9	22.0	15.0	12.0
5-F	11.9	13.8	13.6	5.6	5.3	6.7	19.4	16.2	16.6
6-F	12.7	12.9	12.7	6.3	4.9	7.1	17.6	15.6	13.3
Female Mean	12.4	13.6	12.6	6.0	5.3	6.6	19.7	15.6	14.0
Group Mean	12.1	13.5	12.8	5.8	5.7	6.6	23.4	18.6	18.2

TABLE XIII

Hemoglobin, Erythrocyte, and Total Leukocyte Count

Test Group III

Dog Number and Sex	Hemoglobin, g/100 ml Days: 0	45	90	Erythrocytes, Millions/mm^3 Days: 0	45	90	Leukocytes, Thousands/mm^3 Days: 0	45	90
1-M	11.5	12.3	11.6	6.2	6.6	5.8	11.5	14.5	13.5
2-M	11.6	11.9	12.1	5.5	6.3	6.1	11.6	17.8	17.7
3-M	12.3	13.7	13.3	5.8	6.4	6.8	12.8	17.3	16.2
Male Mean	11.8	12.6	12.3	5.8	6.4	6.2	12.0	16.5	15.9
4-F	11.2	12.2	10.1	5.3	5.3	5.7	11.2	16.3	20.7
5-F	10.7	12.3	12.9	5.5	5.7	6.3	10.7	12.7	16.5
6-F	13.1	12.3	11.9	6.1	6.3	5.9	15.3	13.6	13.9
Female Mean	11.7	12.3	11.6	5.6	5.8	6.0	12.4	14.2	17.0
Group Mean	11.8	12.4	12.0	5.7	6.1	6.1	12.2	15.4	16.4

TABLE XIV

Differential Leukocyte Count

Control Group

Dog Number and Sex	Lymphocytes Days: 0	45	90	Monocytes Days: 0	45	90	Neutrophils Days: 0	45	90	Eosinophils Days: 0	45	90	Basophils Days: 0	45	90
1-M	26	25	23	6	3	7	66	64	66	2	7	4	0	1	0
2-M	28	27	21	7	2	6	62	63	70	3	8	3	0	0	0
3-M	30	28	25	6	4	5	59	61	64	5	7	6	0	0	0
Male Mean	28.0	26.7	23.0	6.7	3.0	6.0	62.3	62.7	66.7	3.3	7.7	4.3	0	0.3	0
4-F	24	27	29	6	7	4	67	62	60	2	3	7	1	1	0
5-F	26	21	25	6	5	4	62	69	63	6	4	8	0	1	0
6-F	29	29	30	5	5	5	59	62	58	6	4	7	1	0	0
Female Mean	26.3	25.7	28.0	5.7	5.7	4.3	62.7	64.3	60.3	4.7	3.7	7.3	0.7	0.7	0
Group Mean	27.2	26.2	25.2	6.2	4.4	5.2	62.5	63.5	63.5	4.0	5.7	5.8	0.4	0.5	0

Number of Cells per Hundred

TABLE XV

Differential Leukocyte Count

Test Group I

Dog Number and Sex	Number of Cells per hundred														
	Lymphocytes Days:			Monocytes Days:			Neutrophils Days:			Eosinophils Days:			Basophils Days:		
	0	45	90	0	45	90	0	45	90	0	45	90	0	45	90
1-M	26	27	25	4	6	3	66	61	67	3	5	5	1	1	0
2-M	25	26	24	6	5	4	65	63	66	4	6	6	0	0	0
3-M	28	29	22	7	4	6	61	60	67	4	7	5	0	0	0
Male Mean	26.3	27.3	23.6	5.6	5.0	4.3	64.0	61.3	66.7	3.7	6.0	5.3	0.3	0.3	0
4-F	27	25	23	5	4	6	62	63	63	6	8	3	0	0	0
5-F	29	26	23	3	2	5	59	64	68	9	6	3	0	2	1
6-F	27	26	23	3	5	6	65	63	66	5	6	5	0	0	0
Female Mean	27.7	25.7	23.0	3.7	3.7	5.7	62.0	63.3	67.3	6.7	6.7	3.7	0.	0.7	0.3
Group Mean	27.0	26.5	23.3	4.6	4.4	5.0	63.0	62.3	67.0	5.2	6.4	4.5	0.2	0.5	0.2

TABLE XVI

Differential Leukocyte Count

Test Group II

Dog Number and Sex	Lymphocytes Days:			Monocytes Days:			Neutrophils Days:			Eosinophils Days:			Basophils Days:		
	0	45	90	0	45	90	0	45	90	0	45	90	0	45	90
1-M	27	26	21	7	7	7	61	61	67	5	6	5	0	0	0
2-M	20	27	25	5	8	8	71	59	62	4	6	5	0	0	0
3-M	24	13	26	7	6	5	60	72	64	7	4	5	2	0	0
Male Mean	23.6	23.7	24.0	6.3	7.0	6.7	64.0	64.0	64.3	5.3	5.3	5.0	0.7	0.0	0.0
4-F	24	23	22	6	7	4	65	67	66	5	3	7	0	0	1
5-F	25	22	26	5	7	3	66	69	63	4	2	7	0	0	1
6-F	25	20	24	7	5	2	63	68	66	5	5	8	0	2	0
Female Mean	24.7	21.7	24.0	6.0	6.3	3.0	64.7	68.0	65.0	4.7	3.3	7.3	0.0	0.7	0.7
Group Mean	24.2	22.7	24.0	6.2	6.7	4.9	64.4	66.0	64.7	5.0	4.3	6.2	0.4	0.4	0.4

Number of Cells per Hundred

TABLE XVII

Differential Leukocyte Count

Test Group III

Number of Cells per Hundred

Dog Number And Sex	Lymphocytes Days:			Monocytes Days:			Neutrophils Days:			Eosinophils Days:			Basophils Days:		
	0	45	90	0	45	90	0	45	90	0	45	90	0	45	90
1-M	17	26	22	7	6	4	73	63	70	3	4	4	0	1	0
2-M	33	28	22	4	4	5	61	66	68	0	2	5	2	0	0
3-M	25	26	26	2	3	2	69	64	69	4	7	3	0	0	0
Male Mean	25.0	26.7	23.3	4.3	4.3	3.7	67.7	64.3	69.0	2.3	4.3	4.0	0.7	0.3	0.0
4-F	26	20	24	1	6	8	72	68	62	1	4	5	0	2	1
5-F	19	21	22	5	7	8	71	70	60	4	2	9	1	0	1
6-F	23	18	21	4	3	7	68	71	62	4	8	9	1	0	1
Female Mean	22.7	19.7	22.3	3.3	5.3	7.7	70.3	70.0	61.3	3.0	4.7	7.7	1.0	0.7	1.0
Group Mean	23.8	23.2	22.8	3.8	4.8	5.7	69.0	67.2	65.2	2.6	4.8	5.8	0.8	0.5	0.5

E. __Urine Analyses__

The results of urine analyses conducted upon all dogs are shown in Table XVIII.

No significant abnormalities were noted.

TABLE XVIII

Urine Analyses

Group	Dog Number and Sex	Reducing Substances Days: 0	45	90	Albumin Days: 0	45	90	Microscopic Elements Days: 0	45	90
	1-M	-	-	-	-	-	-	+	-	-
	2-M	-	-	-	-	-	-	-	-	-
CONTROL	3-M	-	-	-	-	-	-	-	-	-
	4-F	-	+	-	-	+	-	+	+	-
	5-F	-	-	-	-	-	-	-	-	-
	6-F	-	-	-	-	-	-	-	-	-
	1-M	-	-	-	-	-	-	+	-	+
	2-M	-	-	-	+	-	-	-	-	-
I	3-M	-	-	-	-	-	-	-	-	-
	4-F	-	-	-	-	-	-	+	-	-
	5-F	-	+	-	-	-	-	-	+	-
	6-F	-	-	-	-	-	-	-	-	-

- = negative
+ = very slight amounts

072

TABLE XVIII (Continued)

Urine Analyses

Group	Dog Number and Sex	Reducing Substances Days: 0	45	90	Albumin Days: 0	45	90	Microscopic Elements Days: 0	45	90
II	1-M	-	-	-	-	-	+	-	-	+
	2-M	-	-	-	-	-	+	-	-	-
	3-M	-	-	-	-	-	-	-	-	-
	4-F	-	+	-	-	-	-	-	-	-
	5-F	-	-	-	-	-	-	+	-	-
	6-F	-	-	-	-	-	-	-	-	-
III	1-M	-	-	-	-	-	-	+	+	-
	2-M	-	-	-	+	-	-	-	-	-
	3-M	-	-	-	-	-	-	-	-	-
	4-F	-	-	-	-	-	-	-	-	-
	5-F	-	-	-	-	-	-	-	-	+
	6-F	-	-	+	-	-	-	-	-	-

\- = negative
+ = very slight amounts

F. Liver and Kidney Function Tests

The results of the physiological organ function tests performed upon the livers and kidneys of all dogs are presented in Tables XIX and XX respectively.

No evidence of organ dysfunction was noted.

TABLE XIX

Liver Function Tests

Group	Dog Number and Sex	Per Cent Retention of Sulfobromophthalein after 15 Minutes* Days: 0	45	90
	1-M	9	14	14
	2-M	7	8	12
	3-M	10	12	14
Control	Male Mean	9	11	13
	4-F	15	7	12
	5-F	9	15	9
	6-F	9	9	7
	Female Mean	10	10	9
	Group Mean	16	10	11
	1-M	11	12	14
	2-M	8	12	13
	3-M	10	9	14
I	Male Mean	10	11	14
	4-F	7	12	12
	5-F	8	14	7
	6-F	12	10	7
	Female Mean	9	12	9
	Group Mean	10	12	12

* Normal retention 15 minutes after intravenous injection is 7 to 14 per cent.

TABLE XIX (Continued)

Liver Function Tests

Group	Dog Number and Sex	Per Cent Retention of Sulfobromophthalein after 15 Minutes*		
		Days:		
		0	45	90
	1-M	14	9	11
	2-M	13	13	7
	3-M	11	11	10
II	Male Mean	13	11	9
	4-F	11	6	12
	5-F	4	9	13
	6-F	15	12	11
	Female Mean	10	9	12
	Group Mean	12	10	11
	1-M	9	7	7
	2-M	10	13	8
	3-M	7	8	8
III	Male Mean	9	9	8
	4-F	11	8	8
	5-F	14	13	12
	6-F	8	9	7
	Female Mean	11	10	9
	Group Mean	10	10	8

* Normal retention 15 minutes after intravenous injection is 7 to 14 per cent.

TABLE XII

Kidney Function Tests

Group	Animal Number and Sex	Per Cent Excretion After 90 Minutes* Days:		
		0	45	90
Control	1-M	46	48	39
	2-M	52	49	43
	3-M	46	53	41
	Male Mean	47	50	41
	4-F	45	53	43
	5-F	48	54	45
	6-F	49	57	40
	Female Mean	48	55	44
	Group Mean	48	55	42
I	1-M	43	48	49
	2-M	46	56	53
	3-M	42	52	54
	Male Mean	45	52	52
	4-F	49	45	43
	5-F	45	49	49
	6-F	44	54	52
	Female Mean	46	49	48
	Group Mean	46	50	50

* Normal excretion 90 minutes after intramuscular injection is 40 to 60 per cent.

TABLE XX (Continued)

Kidney Function Tests

Group	Animal Number and Sex	Per Cent Excretion After 90 Minutes Days: 0	45	90
	1-M	55	53	43
	2-M	53	50	47
	3-M	49	55	42
II	Male Mean	53	53	44
	4-F	52	46	55
	5-F	56	48	52
	6-F	49	43	49
	Female Mean	52	46	52
	Group Mean	52	50	43
	1-M	51	56	46
	2-M	53	43	52
	3-M	4?	43	46
III	Male Mean	52	49	48
	4-F	45	43	49
	5-F	42	51	48
	6-F	40	41	45
	Female Mean	42	45	47
	Group Mean	47	47	48

* Normal excretion 90 minutes after intramuscular injection is 40 to 60 per cent.

G. Pathology

The tissues and organs taken from all dogs sacrificed at the termination of the study were subjected to both gross and microscopic pathologic examination.

1. Gross Pathology

A summary of the gross pathologic findings is presented in Tables XXI to XXIV.

Gross pathologic findings for test dogs were comparable to those noted among control dogs.

Key:

A. Findings

NPC = No Pathologic Change

B. Grading System

1 = slight

2 = mild

3 = moderate

4 = severe

5 = extreme

TABLE XXI

Gross Pathologic Findings

Control Group

Organs	Findings	Incidence			Average Grade		
		Male	Female	Male & Female	Male	Female	Male & Female
Heart	NPC	3/3	3/3	6/6	0	0	0
Aorta	NPC	3/3	3/3	6/6	0	0	0
Trachea	NPC	3/3	3/3	6/6	0	0	0
Lungs	NPC	2/3	3/3	5/6	0	0	0
	Pneumonitis	1/3	0/3	1/6	1	-	-
Liver	NPC	3/3	3/3	6/6	0	0	0
Gall Bladder	NPC	3/3	3/3	6/6	0	0	0
Pancreas	NPC	3/3	3/3	6/6	0	0	0
Esophagus	NPC	3/3	3/3	6/6	0	0	0
Stomach:							
Cardiac Region	NPC	3/3	3/3	6/6	0	0	0
Fundus Region	NPC	3/3	3/3	6/6	0	0	0
Pyloric Region	NPC	3/3	3/3	6/6	0	0	0
Small Intestine							
Duodenum	NPC	3/3	3/3	6/6	0	0	0
Jejunum	NPC	3/3	3/3	6/6	0	0	0
Ileum	NPC	3/3	3/3	6/6	0	0	0
Colon	NPC	3/3	3/3	6/6	0	0	0
Spleen	NPC	3/3	3/3	6/6	0	0	0
Lymph Nodes	NPC	3/3	3/3	6/6	0	0	0
Kidneys	NPC	3/3	3/3	6/6	0	0	0
Urinary Bladder	NPC	3/3	3/3	6/6	0	0	0
Testes	NPC	3/3	-	-	0	-	-
Ovaries	NPC	-	3/3	-	-	0	-
Prostate	NPC	3/3	-	-	0	-	-
Uterus	NPC	-	3/3	-	-	0	-
Pituitary	NPC	3/3	3/3	6/6	0	0	0
Adrenal Glands	NPC	3/3	3/3	6/6	0	0	0
Submaxillary							
Salivary Glands	NPC	3/3	3/3	6/6	0	0	0
Thyroid Gland	NPC	3/3	3/3	6/6	0	0	0
Parathyroid Glands	NPC	3/3	3/3	6/6	0	0	0
Skeletal Muscle	NPC	3/3	3/3	6/6	0	0	0
Bone	NPC	3/3	3/3	6/6	0	0	0
Peripheral Nerves	NPC	3/3	3/3	6/6	0	0	0
Spinal Cord	NPC	3/3	3/3	6/6	0	0	0
Brain	NPC	3/3	3/3	6/6	0	0	0

TABLE XXII

Gross Pathologic Findings

Test Group I

Organs	Findings	Incidence		-Male & Female	Average Grade		Male & Female
		Male	Female		Male	Female	
Heart	NPC	3/3	3/3	6/6	0	0	0
Aorta	NPC	3/3	3/3	6/6	0	0	0
Trachea	NPC	3/3	3/3	6/6	0	0	0
Lungs	NPC	1/3	3/3	4/6	0	0	0
	Pneumonitis	2/3	0/3	2/6	1	-	-
Liver	NPC	3/3	3/3	6/6	0	0	0
Gall Bladder	NPC	3/3	3/3	6/6	0	0	0
Pancreas	NPC	3/3	3/3	6/6	0	0	0
Esophagus	NPC	3/3	3/3	6/6	0	0	0
Stomach							
Cardiac Region	NPC	3/3	3/3	6/6	0	0	0
Fundus Region	NPC	3/3	3/3	6/6	0	0	0
Pyloric Region	NPC	3/3	3/3	6/6	0	0	0
Small Intestine							
Duodenum	NPC	3/3	3/3	6/6	0	0	0
Jejunum	NPC	3/3	3/3	6/6	0	0	0
Ileum	NPC	3/3	3/3	6/6	0	0	0
Colon	NPC	3/3	3/3	6/6	0	0	0
Spleen	NPC	2/3	3/3	5/6	0	0	0
Lymph Nodes	NPC	3/3	3/3	6/6	0	0	0
Kidneys	NPC	3/3	3/3	6/6	0	0	0
Urinary Bladder	NPC	3/3	3/3	6/6	0	0	0
Testes	NPC	3/3	-	-	0	-	-
Ovaries	NPC	-	3/3	-	-	0	-
Prostate	NPC	3/3	-	-	0	-	-
Uterus	NPC	-	3/3	-	-	0	-
Pituitary	NPC	3/3	2/3	6/6	0	0	0
Adrenal Gland	NPC	3/3	3/3	6/6	0	0	0
Submaxillary							
Salivary Glands	NPC	3/3	3/3	6/6	0	0	0
Thyroid Gland	NPC	3/3	3/3	6/6	0	0	0
Parathyroid Glands	NPC	3/3	3/3	6/6	0	0	0
Skeletal Muscle	NPC	3/3	3/3	6/6	0	0	0
Bone	NPC	3/3	3/3	6/6	0	0	0
Peripheral Nerves	NPC	3/3	3/3	6/6		0	0
Spinal Cord	NPC	3/3	3/3	6/6	0	0	0
Brain	NPC	3/3	3/3	6/6	0	0	0

TABLE XXIII

Gross Pathologic Findings

Test Group II

Organs	Findings	Incidence Male	Incidence Female	Incidence Male & Female	Average Grade Male	Average Grade Female	Average Grade Male & Female
Heart	NPC	3/3	3/3	6/6	0	0	0
Aorta	NPC	3/3	3/3	6/6	0	0	0
Trachea	NPC	3/3	3/3	6/6	0	0	0
Lungs	NPC	3/3	3/3	6/6	0	0	0
Liver	NPC	3/3	3/3	6/6	0	0	0
Gall Bladder	NPC	3/3	3/3	6/6	0	0	0
Pancreas	NPC	3/3	3/3	6/6	0	0	0
Esophagus	NPC	3/3	3/3	6/6	0	0	0
Stomach							
Cardiac Region	NPC	3/3	3/3	6/6	0	0	0
Fundus Region	NPC	3/3	3/3	6/6	0	0	0
Pyloric Region	NPC	3/3	3/3	6/6	0	0	0
Small Intestine							
Duodenum	NPC	3/3	3/3	6/6	0	0	0
Jejunum	NPC	3/3	3/3	6/6	0	0	0
Ileum	NPC	3/3	3/3	6/6	0	0	0
Colon	NPC	3/3	3/3	6/6	0	0	0
Spleen	NPC	2/3	3/3	6/6	0	0	0
Lymph Nodes	NPC	3/3	3/3	6/6	0	0	0
Kidneys	NPC	3/3	3/3	6/6	0	0	0
Urinary Bladder	NPC	3/3	3/3	6/6	0	0	0
Testes	NPC	3/3	-	-	0	-	-
Ovaries	NPC	-	3/3	-	-	0	-
Prostate	NPC	3/3	-	-	0	-	-
Uterus	NPC	-	3/3	-	-	0	-
Pituitary	NPC	3/3	3/3	6/6	0	0	0
Adrenal Gland	NPC	3/3	3/3	6/6	0	0	0
Submaxillary							
Salivary Glands	NPC	3/3	3/3	6/6	0	0	0
Thyroid Gland	NPC	3/3	3/3	6/6	0	0	0
Parathyroid	NPC	3/3	3/3	6/6	0	0	0
Skeletal Muscle	NPC	3/3	3/3	6/6	0	0	0
Bone	NPC	3/3	3/3	6/6	0	0	0
Peripheral Nerves	NPC	3/3	3/3	6/6	0	0	0
Spinal Cord	NPC	3/3	3/3	6/6	0	0	0
Brain	NPC	3/3	3/3	6/6	0	0	0

TABLE XXIV

Gross Pathologic Findings

Test Group III

Organs	Findings	Incidence Male	Female	Male & Female	Average Grade Male	Female	Male & Female
Heart	NPC	3/3	3/3	6/6	0	0	0
Aorta	NPC	3/3	3/3	6/6	0	0	0
Trachea	NPC	3/3	3/3	6/6	0	0	0
Lungs	NPC	2/3	3/3	5/6	0	0	0
	Pneumonitis	1/3	0/3	1/6	1	-	-
Liver	NPC	3/3	3/3	6/6	0	0	0
Gall Bladder	NPC	3/3	3/3	6/6	0	0	0
Pancreas	NPC	3/3	3/3	6/6	0	0	0
Esophagus	NPC	3/3	3/3	6/6	0	0	0
Stomach							
Cardiac Region	NPC	3/3	3/3	6/6	0	0	0
Fundus Region	NPC	3/3	3/3	6/6	0	0	0
Pyloric Region	NPC	3/3	3/3	6/6	0	0	0
Small Intestine							
Duodenum	NPC	3/3	3/3	6/6	0	0	0
Jejunum	NPC	3/3	3/3	6/6	0	0	0
Ileum	NPC	3/3	3/3	6/6	0	0	0
Colon	NPC	3/3	3/3	6/6	0	0	0
Spleen	NPC	2/3	3/3	5/6	0	0	0
	Hyperemia	1/3	0/3	1/6	2	-	-
Lymph Nodes	NPC	3/3	3/3	6/6	0	0	0
Kidneys	NPC	3/3	3/3	6/6	0	0	0
Urinary Bladder	NPC	3/3	3/3	6/6	0	0	0
Testes	NPC	3/3	-	-	0	-	-
Ovaries	NPC	-	3/3	-	-	0	-
Prostate	NPC	3/3	-	-	0	-	-
Uterus	NPC	-	3/3	-	-	0	-
Pituitary	NPC	3/3	3/3	6/6	0	0	0
Adrenal Glands	NPC	3/3	3/3	6/6	0	0	0
Submaxillary							
Salivary Glands	NPC	3/3	3/3	6/6	0	0	0
Thyroid Gland	NPC	3/3	3/3	6/6	0	0	0
Parathyroid Glands	NPC	3/3	3/3	6/6	0	0	0
Skeletal Muscle	NPC	3/3	3/3	6/6	0	0	0
Bone	NPC	3/3	3/3	6/6	0	0	0
Peripheral Nerves	NPC	3/3	3/3	6/6	0	0	0
Spinal Cord	NPC	3/3	3/3	6/6	0	0	0
Brain	NPC	3/3	3/3	6/6	0	0	0

2. Microscopic Pathology

A summary of the histopathologic findings is presented in Tables XXV to XXVIII.

Histopathologic findings for test dogs were comparable to those noted among control dogs.

TABLE XIV

Histopathologic Findings

Control Group

Organs	Findings	Incidence			Average Grade		
		Male	Female	Male & Female	Male	Female	Male & Female
Heart	NPC	3/3	3/3	6/6	0	0	0
Aorta	NPC	3/3	3/3	6/6	0	0	0
Trachea	NPC	3/3	3/3	6/6	0	0	0
Lung	NPC	2/3	3/3	5/6	0	0	0
	Hyperemia	1/3	0/3	1/6	3	-	-
	Pneumonitis	1/3	0/3	1/6	1	-	-
Liver	NPC	1/3	1/3	2/6	0	0	0
	Hyperemia	2/3	2/3	4/6	1	1	1
	Pericholangitis	2/3	1/3	3/6	1	1	1
Gall Bladder	NPC	3/3	3/3	6/6	0	0	0
Pancreas	NPC	3/3	3/3	6/6	0	0	0
Esophagus	NPC	3/3	3/3	6/6	0	0	0
Stomach							
Cardiac Region	NPC	3/3	3/3	6/6	0	0	0
Fundus Region	NPC	3/3	3/3	6/6	0	0	0
Pyloric Region	NPC	3/3	3/3	6/6	0	0	0
Small Intestine							
Duodenum	NPC	3/3	3/3	6/6	0	0	0
Jejunum	NPC	3/3	3/3	6/6	0	0	0
Ileum	NPC	3/3	3/3	6/6	0	0	0
Colon	NPC	3/3	3/3	6/6	0	0	0
Spleen	NPC	1/3	1/3	2/6	0	0	0
	Hyperemia	2/3	2/3	4/6	1	1	1
Lymph Node	NPC	3/3	3/3	6/6	0	0	0
Kidney	NPC	3/3	3/3	6/6	0	0	0
Urinary Bladder	NPC	3/3	3/3	6/6	0	0	0
Testis	NPC	3/3	-	-	0	-	-
Ovary	NPC	-	3/3	-	-	0	-
Prostate	NPC	3/3	-	-	0	-	-
Uterus	NPC	-	3/3	-	-	0	-
Pituitary	NPC	3/3	3/3	6/6	0	0	0
Adrenal Gland	NPC	3/3	3/3	6/6	0	0	0
Submaxillary Salivary Gland	NPC	3/3	3/3	6/6	0	0	0
Thyroid Gland	NPC	3/3	3/3	6/6	0	0	0

TABLE XXV (Continued)

Histopathologic Findings

Control Group

		Incidence			Average Grade		
Organs	Findings	Male	Female	Male & Female	Male	Female	Male & Female
Parathyroid Gland	NPC	3/3	3/3	6/6	0	0	0
Skeletal Muscle	NPC	3/3	3/3	6/6	0	0	0
Bone Marrow	NPC	3/3	3/3	6/6	0	0	0
Peripheral Nerve	NPC	3/3	3/3	6/6	0	0	0
Spinal Cord	NPC	3/3	3/3	6/6	0	0	0
Brain: Cerebrum	NPC	3/3	3/3	6/6	0	0	0
Cerebellum	NPC	3/3	3/3	6/6	0	0	0
Pons	NPC	3/3	3/3	6/6	0	0	0

TABLE XLVI

Histopathologic Findings

Test Group I

		Incidence			Average Grade		
Organ	Finding	Male	Female	Male & Female	Male	Female	Male & Female
Heart	NPC	3/3	3/3	6/6	0	0	0
Aorta	NPC	3/3	3/3	6/6	0	0	0
Trachea	NPC	3/3	3/3	6/6	0	0	0
Lung	NPC	3/3	3/3	6/6	0	0	0
Liver	NPC	1/3	1/3	2/6	0	0	0
	Hyperemia	2/3	2/3	4/6	1	1	1
	Pericholangitis	1/3	2/3	3/6	1	1	1
Gall Bladder	NPC	3/3	3/3	6/6	0	0	0
Pancreas	NPC	3/3	3/3	6/6	0	0	0
Esophagus	NPC	3/3	3/3	6/6	0	0	0
Stomach							
Cardiac Region	NPC	3/3	3/3	6/6	0	0	0
Fundus Region	NPC	3/3	3/3	6/6	0	0	0
Pyloric Region	NPC	3/3	3/3	6/6	0	0	0
Small Intestine							
Duodenum	NPC	3/3	3/3	6/6	0	0	0
Jejunum	NPC	3/3	3/3	6/6	0	0	0
Ileum	NPC	3/3	3/3	6/6	0	0	0
Colon	NPC	3/3	3/3	6/6	0	0	0
Spleen	NPC	1/3	1/3	2/6	0	0	0
	Hyperemia	2/3	2/3	4/6	1	1	1
Lymph Node	NPC	3/3	3/3	6/6	0	0	0
Kidney	NPC	3/3	3/3	6/6	0	0	0
Urinary Bladder	NPC	3/3	3/3	6/6	0	0	0
Testis	NPC	3/3	-	-	0	-	-
Ovary	NPC	-	3/3	-	-	0	-
Prostate	NPC	3/3	-	-	0	-	-
Uterus	NPC	-	3/3	-	-	0	-
Pituitary	NPC	3/3	3/3	6/6	0	0	0
Adrenal Gland	NPC	3/3	3/3	6/6	0	0	0
Submaxillary Salivary Gland	NPC	3/3	3/3	6/6	0	0	0
Thyroid	NPC	3/3	3/3	6/6	0	0	0
Parathyroid	NPC	3/3	3/3	6/6	0	0	0
Skeletal Muscle	NPC	3/3	3/3	6/6	0	0	0
Bone Marrow	NPC	3/3	3/3	6/6	0	0	0
Peripheral Nerve	NPC	3/3	3/3	6/6	0	0	0
Spinal Cord	NPC	3/3	3/3	6/6	0	0	0
Brain: Cerebrum	NPC	3/3	3/3	6/6	0	0	0
Cerebellum	NPC	3/3	3/3	6/6	0	0	0
Pons	NPC	3/3	3/3	6/6	0	0	0

TABLE XXVII

Histopathologic Findings

Test Group II

Organs	Findings	Incidence			Average Grade		
		Male	Female	Male & Female	Male	Female	Male & Female
Heart	NPC	3/3	3/3	6/6	0	0	0
Aorta	NPC	3/3	3/3	6/6	0	0	0
Trachea	NPC	3/3	3/3	6/6	0	0	0
Lung	NPC	3/3	3/3	6/6	0	0	0
Liver	NPC	1/3	2/3	3/6	0	0	0
	Hyperemia	2/3	1/3	3/6	1	1	1
	Perichol-angitis	2/3	1/3	3/6	1	1	1
Gall Bladder	NPC	3/3	3/3	6/6	0	0	0
Pancreas	NPC	3/3	3/3	6/6	0	0	0
Esophagus	NPC	3/3	3/3	6/6	0	0	0
Stomach							
Cardiac Region	NPC	3/3	3/3	6/6	0	0	0
Fundus Region	NPC	3/3	3/3	6/6	0	0	0
Pyloric Region	NPC	3/3	3/3	6/6	0	0	0
Small Intestine							
Duodenum	NPC	3/3	3/3	6/6	0	0	0
Jejunum	NPC	3/3	3/3	6/6	0	0	0
Ileum	NPC	3/3	3/3	6/6	0	0	0
Colon	NPC	3/3	3/3	6/6	0	0	0
Spleen	NPC	1/3	1/3	2/6	0	0	0
	Hyperemia	2/3	2/3	4/6	1	1	1
Lymph Node	NPC	3/3	3/3	6/6	0	0	0
Kidney	NPC	3/3	2/3	5/6	0	0	0
	Interstitial Inflammation	0/3	1/3	1/6	-	1	-
Urinary Bladder	NPC	3/3	3/3	6/6	0	0	0
Testis	NPC	3/3	-	-	0	-	-
Ovary	NPC	-	3/3	-	-	0	-
Prostate	NPC	3/3	-	-	0	-	-
Uterus	NPC	-	3/3	-	-	0	-
Pituitary	NPC	3/3	3/3	6/6	0	0	0
Submaxillary Salivary Gland	NPC	3/3	3/3	6/6	0	0	0
Thyroid Gland	NPC	3/3	3/3	6/6	0	0	0
Parathyroid Gland	NPC	3/3	3/3	6/6	0	0	0
Skeletal Muscle	NPC	3/3	3/3	6/6	0	0	0
Bone Marrow	NPC	3/3	3/3	6/6	0	0	0
Peripheral Nerve	NPC	3/3	3/3	6/6	0	0	0
Spinal Cord	NPC	3/3	3/3	6/6	0	0	0
Brain: Cerebrum	NPC	3/3	3/3	6/6	0	0	0
Cerebellum	NPC	3/3	3/3	6/6	0	0	0
Pons	NPC	3/3	3/3	6/6	0	0	0

TABLE XLVIII

Histopathologic Findings

Test Group III

		Incidence		Male &	Average Grade		Male &
Organs	Findings	Male	Female	Female	Male	Female	Female
Heart	NPC	3/3	3/3	6/6	0	0	0
Aorta	NPC	3/3	3/3	6/6	0	0	0
Trachea	NPC	3/3	3/3	6/6	0	0	0
Lung	NPC	3/3	2/3	5/6	0	0	0
	Hyperemia	0/3	1/3	1/6	-	3	-
	Pneumonitis	0/3	1/3	1/6	-	1	-
Liver	NPC	1/3	1/3	2/6	0	0	0
	Hyperemia	2/3	2/3	4/6	1	1	1
	Pericholangitis	2/3	1/3	3/6	1	1	1
Gall Bladder	NPC	3/3	3/3	6/6	0	0	0
Pancreas	NPC	3/3	3/3	6/6	0	0	0
Esophagus	NPC	3/3	3/3	6/6	0	0	0
Stomach							
Cardiac Region	NPC	3/3	3/3	6/6	0	0	0
Fundus Region	NPC	3/3	3/3	6/6	0	0	0
Pyloric Region	NPC	3/3	3/3	6/6	0	0	0
Small Intestine							
Duodenum	NPC	3/3	3/3	6/6	0	0	0
Jejunum	NPC	3/3	3/3	6/6	0	0	0
Ileum	NPC	3/3	3/3	6/6	0	0	0
Colon	NPC	3/3	3/3	6/6	0	0	0
Spleen	NPC	1/3	2/3	3/6	0	0	0
	Hyperemia	2/3	1/3	3/6	1	1	1
Lymph Node	NPC	3/3	3/3	6/6	0	0	0
Kidney	NPC	3/3	3/3	6/6	0	0	0
Urinary Bladder	NPC	3/3	3/3	6/6	0	0	0
Testis	NPC	3/3	-	-	0	-	-
Ovary	NPC	-	3/3	-	-	0	-
Prostate	NPC	3/3	-	-	0	-	-
Uterus	NPC	-	3/3	-	-	0	-
Pituitary	NPC	3/3	3/3	6/6	0	0	0
Adrenal Gland	NPC	3/3	3/3	6/6	0	0	0
Submaxillary							
Salivary Gland	NPC	3/3	3/3	6/6	0	0	0
Thyroid Gland	NPC	3/3	3/3	6/6	0	0	0

TABLE XXVII (Continued)

Histopathologic Findings

Test Group III

Organs	Findings	Incidence		Male &	Average Grade		Male &
		Male	Female	Female	Male	Female	Female
Parathyroid Gland	NPC	3/3	3/3	6/6	0	0	0
Skeletal Muscle	NPC	3/3	3/3	6/6	0	0	0
Bone Marrow	NPC	3/3	3/3	6/6	0	0	0
Peripheral Nerve	NPC	3/3	3/3	6/6	0	0	0
Spinal Cord	NPC	3/3	3/3	6/6	0	0	0
Brain: Cerebrum	NPC	3/3	3/3	6/6	0	0	0
Cerebellum	NPC	3/3	3/3	6/6	0	0	0
Pons	NPC	3/3	3/3	6/6	0	0	0

IV. Summary

A. Body Weights

The results of a 90-day subacute oral toxicity investigation in pure bred beagle hounds, employing Sample A at three graded dietary levels did not disclose any adverse effects upon normal growth patterns.

B. Food Consumption

All of the dogs in the test groups exhibited food consumption comparable to or greater than the control dogs throughout the investigation.

C. Mortality and Reactions

There were no mortalities or untoward reactions recorded during the 90-day investigational period.

D. Hematologic Studies and Urine Analyses

The results of complete hematologic studies and urine analyses conducted periodically upon all dogs were normal.

E. Liver and Kidney Function Tests

The results of periodic liver and kidney function tests did not disclose any evidence of organ dysfunction.

F. Pathology

1. Gross Pathologic Findings

Complete autopsy of all dogs sacrificed at the termination of the study did not reveal any significant gross pathologic change.

2. Microscopic Pathologic Findings

Histologic Findings for the tissues and organs taken from

the dogs in all of the test groups were found to be comparable to controls. No microscopic changes were seen in the test animals which could be correlated with ingestion of the test material.

Respectfully submitted,

INDUSTRIAL BIO-TEST LABORATORIES, INC.

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Report approved by: J. C. Calandra, M.D., Ph.D.
Director

January 22, 1962

United States Patent Office

2,762,709
Patented Sept. 11, 1956

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2,762,709

TREATING METHOD FOR POTATOES

Martin A. Janis, Maumee, and Ernest G. Bremforder, Toledo, Ohio, assignors to Kuehmann Foods, Inc., Toledo, Ohio, a corporation of Ohio

Application May 19, 1953, Serial No. 356,062

6 Claims. (Cl. 99—100)

This invention relates to a treating method for potatoes and, more particularly, to a method especially suited for the treatment prior to frying of potatoes to prevent or minimize caramelizing during frying.

The problem of discoloration of potatoes in the course of deep fat frying has been encountered for a considerable period of time, and numerous attempts have been made to avoid this difficulty.

During storage of potatoes a chemical or enzymatic reaction occurs in the course of which starch in potatoes is converted to reducing sugars. Potatoes stored at extremely low temperatures, for example near 40° F., show a gradual increase in reducing sugar content. Accordingly, potatoes stored at such low temperatures for considerable periods of time have a substantial reducing sugar content. It has been demonstrated that such reducing sugar "caramelizes" when the potatoes are fried, and that such caramelizing is responsible for a relatively dark brown color in potatoes. Such dark brown or "caramelized" cooked potato products have a taste that has been considered unpleasant by consumers of potato products. Furthermore, the industries that supply deep fat fried potato products such as potato chips, julienne potatoes, and the like, have built trade acceptance on a light golden-brown color. Accordingly, consumers and distributors refuse deep fat fried potato products having the dark brown color characteristic of caramelization solely because of the color, and quite apart from any objectionable taste.

If potatoes are stored at only a moderately low temperature, in such manner that air is free to circulate around the potatoes, and under controlled humidity conditions, the reducing sugar content of potatoes can be lowered somewhat. Apparently, under such conditions, sugar is consumed in the potatoes by enzymatic or metabollic reactions at a rate faster than it is produced by chemical or enzymatic reactions. Accordingly, potato chip manufacturers, for example, have developed the practice of careful storage of potato stocks to control the sugar content therein. Such procedure is usually satisfactory, and enables manufacturers to produce and market potato chips and similar products of a uniform golden-brown color. However, with potatoes of some types such aging techniques are relatively unsatisfactory, and, when a manufacturer's supply of cured potatoes is exhausted it is sometimes necessary for him to replace his raw potato stocks from the open market. Frequently, potatoes available on the open market have been stored at extremely low temperatures with the result that the sugar content thereof is so high that it is impractical or impossible, by subsequent curing, to produce uncaramelized, deep fat fried potato products. For example, during times of potato shortages various manufacturers have found themselves confronted with the choice of closing their plants or using potatoes having such a high content of reducing sugar that only caramelized deep fat fried products can be produced.

In addition to the curing processes discussed above, various attempts have been made to control the reducing sugar content of potatoes by different manipulative operations. For example, the use of a hot alkaline earth salt solution has been suggested as a leaching bath for removing reducing sugars from potatoes; the claim is made for the use of such bath that it removes the sugars without any deleterious effect upon the taste of the finished product, but this claim has not been substantiated in practice. In addition, the use of dilute solutions of such acids as hydrochloric, acetic, lactic, and others, sodium bisulphite solutions and similar leaching baths have also been suggested. However, although highly satisfactory results from the standpoint of color control of the finished product have been achieved, none of the processes heretofore suggested, so far as is known, has had any commercial acceptance because each of such processes imparts a noticeable and undesirable taste to fried potatoes, particularly to deep fat fried potatoes.

Attempts to remove reducing sugars from potatoes with hot water, alone, have also been reported. For example, U. S. Patent 2,418,519 suggests a blanching tank in which water is heated by steam coils, and through which, beneath the water level, potato slices are moved on a conveyor. A pump is provided to withdraw heated water from one end of the blanching tank and to supply this water to the other end thereof and thereby to cause a flow of water in the tank in the direction of potato slice movement therethrough. We have experimented with a treating process substantially identical with that suggested by this patent, and also with one where fresh water was continuously admixed with the blanching water and the temperature maintained by steam coils positioned in the blanching water, and have found that caramelization upon deep fat frying may be substantially decreased by treating potato slices in either of these ways; however, such processes are without commercial application because the potato slices, after deep fat frying, have an "off" or "fishy" taste which renders them unsalable.

Similarly, U. S. Patent 2,448,152 reports attempts to control caramelization upon deep fat frying by treatment of potato slices with "plain hot water." The patent states (column 3, lines 31 et seq.):

"These resulted in removing some of the browning reactants but also resulted in dissolving out a portion of the pectic substances with a resulting destruction of flavor. A lower temperature with plain water had no apparent result on removal of the browning reactants and an increase in time and temperature resulted in 'cooked' taste which was not desirable in the resulting chips."

This report seems to indicate that tests consisting of treatment for an undisclosed time in water at three unstated temperatures of potato slices failed to develop a satisfactory hot water treatment for potato slices.

Further, an article by Whiteman in Potato Chipper, vol. 11, No. 3, October 1951, pages 24, 26, 28, 30 and 32, reports work on hot water treatment of potato slices. The experimental procedure employed is summarized on page 28, column 1, lines 18, et seq., as follows:

"Table 1 shows that potato slices soaked for 1 minute in tap water at 145° F. produced chips of a better color than those washed for a few seconds in tap water at 50° to 60°. The hot water treatment is used commercially to some extent."

By reference to Table 1 it is ascertained that in only one of the tests did such treatment of potato slices for one minute in water at a temperature of 145° F. result in a product that could be deep fat fried to an acceptable color.

The only suggestion in any of these three specifically identified references of a commercial process for subjecting potato slices to a hot water treatment is that from

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Patent 2,418,519, which is not satisfactory because of the "off" or "fishy" taste, after frying, which results therefrom.

So far as is known, no suggested way of removing reducing sugars which undergo the described caramelizing in the course of deep fat frying (aside from the curing processes on the whole, unpeeled, potatoes) has heretofore been commercially acceptable. Therefore, in order to produce marketable foodstuffs manufacturers of deep fat fried potato products have been required to arrange, in some way, that the raw potatoes, prior to peeling, have a sugar content within the range in which caramelizing does not occur. If a given supply of potatoes had an excess of sugar which could not be removed by the described curing process, manufacturers have been unable to produce satisfactory products from that supply.

The present invention is based upon the discovery of a novel way for removing reducing sugars from potatoes, according to a process that has no deleterious effect upon the taste of foodstuffs made from such potatoes.

It is therefore an object of the invention to provide a method for treating potatoes to lower the reducing sugar content thereof to a point where deep fat frying is possible without objectionable caramelizing, and whereby no undesirable taste is imparted to the potatoes.

Other objects and advantages will be apparent from the description which follows, and from the attached drawings, in which:

Fig. 1 is a schematic flow diagram representing preferred conditions for practicing the novel method of the invention; and

Fig. 2 is a plot showing time-temperature relationships for satisfactory treatment of potatoes according to the invention.

According to the invention a method for treating potato slices to prevent caramelizing during subsequent deep fat frying is provided. The method of the invention comprises passing potato slices through and from a treating zone containing hot blanching water at a temperature from about 145° F. to about 160° F. at a rate such that the time of contact between the slices and the blanching water is for from about 1½ minutes to about 8 minutes. Fresh hot water is admixed with the blanching water at a rate of at least ⅒ gallon per hour per pound of potato slices passed through the treating zone which rate is sufficient to maintain the latter at a temperature such that this temperature and the time of contact between the slices and the blanching water are represented by a point within the shaded portion of Fig. 2 of the drawings. Blanching water tailings are withdrawn from the treating zone at approximately the rate that fresh water is added thereto.

Certain experimentally observed facts suggest a theoretical explanation as to why the method of the invention is effective for preventing or minimizing caramelization upon subsequent deep fat frying of potato slices but avoids the "off" or cooked taste heretofore encountered. These facts and the theoretical explanation are here presented solely for the purpose of further illustrating and disclosing the invention, and are in no way to be construed as limitations thereupon.

It has been observed that blanching of potato slices can be accomplished in water heated by steam coils substantially as disclosed in U. S. Patent 2,418,519, but that the blanched potato slices, after deep fat frying, have an "off" or fishy taste, as previously discussed. The discovery of the method of the invention suggests that the "off" or fishy taste is attributable to chemical or enzymatic reaction involving ingredients leached from the potato slices, which reaction occurs at the temperature of the steam coils. Accordingly, the method of the invention avoids such "off" or fishy taste by admixing fresh hot water, preferably continuously, with the blanching water and withdrawing blanching water tailings at approximately the rate that fresh water is so admixed. Such

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admixture of fresh hot water is believed to avoid the "off" or fishy taste by eliminating the need for steam coils around which such chemical or enzymatic reaction as aforesaid seems to occur. Thus for example, when blanching water was maintained at about 153° F. by admixing therewith fresh hot water at a temperature of about 200° F. and at a rate of approximately ½ gallon per hour per gallon of blanching water, no off or fishy taste was imparted to potato slices treated therein at a rate of about 2 pounds per hour per gallon of blanching water (see Example 1).

It has been found experimentally that blanching water temperatures above about 160° F. or below about 145° F. are unsatisfactory for practicing the method of the invention. It is known that when potato slices are immersed in hot water both a leaching action and a cooking action occur. Presumably, the rate of each of these actions is some direct function of temperature, so that each occurs at a faster rate at a higher temperature. The leaching action may remove reducing sugars, assumed herein to be responsible for caramelization, or desirable potato constituents, or both; the cooking and the latter leaching action impair the taste of deep fat fried potato products. In this connection, we have observed that a four minute immersion, a ten minute immersion, or a twelve minute immersion of potato slices in blanching water at a temperature of about 135° F. has no appreciable effect on caramelization on subsequent deep fat frying. However, some impairment of flavor in potato chips produced from slices treated even for ten minutes in water at about 135° F. was noted. Likewise, either a 28 second immersion in blanching water at a temperature of about 190° F. or a 56 second immersion at about 175° F. has no appreciable effect on caramelization, but results in either cooking or removal of desirable substances to such extent that the flavor of the slices, after frying, is impaired.

The experimental facts set forth in the foregoing paragraph are believed to indicate that blanching water at a temperature of about 135° F. is unsatisfactory for treating potato slices according to the invention because at such temperature removal of reducing sugars is at so low a rate that either cooking or removal of desirable potato constituents, probably the latter, is the predominating action. As a consequence, it is impossible appreciably to effect caramelization by treating potatoes in water at such temperature without also producing a product that has been made less palatable by such treatment. Similarly, blanching water at a temperature of 175° F. or 190° F. is unsatisfactory because either cooking or removal of desirable potato constituents, probably the former, proceeds at so high a rate as to be the predominating action. Therefore, it is believed that when potato slices are treated in blanching water at a temperature lower than about 145° F. leaching of desirable constituents is the predominating action over cooking and leaching of reducing sugars. When potato slices are treated in blanching water at a temperature higher than about 160° F. cooking is believed to be the predominating action, over leaching either of reducing sugars or of desirable potato constituents. Accordingly, treating of potato slices by the invention to prevent caramelization upon subsequent deep fat frying without appreciable impairment of flavor must be in water at a temperature from about 145° F. to about 160° F. Also, as previously stated, fresh hot water must be admixed with the blanching water to maintain such temperature, and blanching water tailings must be withdrawn, in order to avoid an off or fishy taste.

Referring now more particularly to the drawings, a preferred embodiment of the invention is represented in Fig. 1 which shows a treating zone containing a volume of water at a temperature from about 150° F. to about 160° F., and shows the flow of potato slices to and from the treating zone, of fresh hot water to the treating zone, and of blanching water tailings from the treating zone.

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It will be apparent that the rate at which undesirable constituents increase in concentration in a volume of blanching water at a given temperature depends upon the rate at which potato slices are treated in such blanching water, and the volume of blanching water employed. As a practical matter it is usually preferred that the volume of blanching water amount to at least about ⅒ gallon per pound per hour of potato slices being treated. When using such a volume of blanching water it has been found that undesirable build up of materials leached from potato slices can be avoided by mixing fresh water with the blanching water at a rate of at least about ⅒ gallon per hour per pound per hour of potato slices being treated. Preferably, the volume of blanching water is from ⅓ to 3 gallons per pound per hour of potato slices being treated, and the rate at which fresh water is admixed therewith is from ⅓ to 1 gallon per hour per pound of potato slices per hour being treated.

These relationships are all represented on Fig. 1 of the drawings, which show a blanching water temperature from about 150° F. to about 160° F., and a preferred residence time of potato slices in the treating zone of from about 2 minutes to about 3 and ½ minutes. Under generally these operating conditions it has been found that if the temperature of the fresh water being added to the treating zone is about 200° F. the blanching water temperature can be maintained within the desired range solely by additions of such fresh hot water, and at approximately ⅓ the rate in gallons per hour that potato slices in pounds per hour are being treated.

Referring now more particularly to Fig. 2, the relatively narrow range of blanching water temperatures, related to times of treatment, operable for treating potato slices according to the invention is represented by the shaded portion thereof. It will be noted by reference to Fig. 2 that temperatures above about 160° F., or below about 145° F. are not satisfactory for treating potatoes according to the invention, and even that temperatures between about 145° F. and about 160° F. are not suitable for such treatment unless the time of contact between the blanching water and the potato slices is controlled within the proper limits. For example, at a blanching water temperature of about 160° F. treatment according to the invention can be accomplished at contact times ranging from about 1½ minutes to about 4 minutes, while at a blanching water temperature of about 150° F. contact times ranging from about 2 minutes to about 4½ minutes are operable, and at blanching water temperatures of about 145° F. contact times ranging from about 5 minutes to about 8 minutes are operable. As has been discussed above, undesirable actions predominate (cooking or leaching of desirable potato constituents) when blanching water temperatures and contact times appreciably outside the range indicated in Fig. 2 are employed.

Although, as indicated in the preceding paragraph, satisfactory treatment of potato slices according to the invention can be accomplished at temperatures between about 145° F. and about 150° F., some relatively slight and usually inconsequential impairment of flavor may result in this temperature range even at the treating times indicated at Fig. 2. Accordingly, it is usually preferred that the blanching water temperature be maintained between about 150° F. and about 160° F., and that the treating time be within the range indicated for operation at such temperatures in Fig. 2. Most desirably, the blanching water temperature is from about 150° F. to about 160° F., and the contact time is from about 2 minutes to about 3½ minutes. For optimum operation with most varieties of potatoes the blanching water temperature should be from about 150° F. to about 160° F., and the treating time should be from about 2½ minutes to about 3 minutes. Under the most preferred and optimum operating conditions maximum leaching of reducing sugars with minimal, if any, removal of desired constituents is accomplished. No deterioration in flavor of

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potato chips, for example, fried from potato slices treated according to the most preferred or optimum conditions is detectable.

The precise relationships between blanching water temperature and treating time most advantageous for a particular lot of potatoes depends upon the potato variety, the type of soil in which it was grown, the length of time the particular lot of potatoes has been stored, and the storage temperatures. The precise treating conditions to be used with a given lot of potatoes should be determined in advance of treatment by running a sample therefrom at a given temperature and contact time and determining whether too much or too little leaching of reducing sugars has occurred. Suitable adjustments in contact time or blanching water temperature can then be made so that potato slices from that lot of potatoes, after deep fat frying, will have the desired color. In determining the treating time and the blanching water temperature to be used, it should be borne in mind that operation outside the ranges represented by Fig. 2 of the drawings will result in some impairment of the flavor of the potato products, as a result either of cooking or undesired leaching in the course of the treating operation. Accordingly, curing of a given lot of potatoes to lower the reducing sugar content, as hereinbefore discussed, is preferable to treating such potato slices at a temperature above about 160° F., or for a time longer, at a given temperature, than represented as satisfactory for such temperature according to Fig. 2 of the drawings.

It has been found experimentally that grease retention in, for example, potato chips produced by deep fat frying of potato slices treated according to the invention can be controlled within certain limits by varying a subsequent operation. It is customary in processing potato slices preparatory to deep fat frying to subject such slices to the action of water sprays just before introduction thereof into the fat. It has been found that by spraying relatively cold water, for example water directly from a main at a temperature of about 40° F., over potato slices treated according to the invention a treated potato slice can be produced which has minimal retention of fat after frying, for example about 33 per cent. However, if water at a temperature of about 185° F., for example, is sprayed over such treated potato slices, just prior to deep fat frying, the resulting fried product has a substantially higher residual fat content, for example in the vicinity of 36 per cent. Accordingly, a further preferred embodiment of the invention involves the spraying of water over potato slices treated according to the invention, and regulation of the temperature of the water so sprayed in order to control within desired limits the retention of fat by such slices in the course of frying.

The term "potato slices" is used herein, and in the appended claims, to include all usual forms of slices, such as those normally employed for producing potato chips, julienne potatoes, shoestring potatoes, French fried potatoes, and the like.

The following example is presented solely for the purpose of further illustrating and disclosing the method of the invention and is not in any way to be construed as a limitation thereon.

Example 1

Potato slices were treated to prevent caramelization upon subsequent deep fat frying according to the following procedure:

A blanching tank having a capacity of approximately 1600 gallons was provided with a substantially horizontal conveyor running generally parallel to the bottom thereof. The blanching tank was also provided with a lift conveyor for receiving potato slices at the discharge end of the generally horizontal conveyor, and for lifting them from the blanching tank. A charge of approximately 1150 gallons of water at a temperature of about 153° F. was added to the blanching tank, and a flow of fresh hot water at a temperature of about 210° F. into the tank at a

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rate of approximately 500 gallons per hour was started. Removal of water from the tank at a rate of approximately 500 gallons per hour was initiated. Movement of the horizontal conveyor and of the lifting conveyor were then started, and the rate of travel adjusted to provide for potato slices a residence time in the blanching tank of approximately 165 seconds. Potato slices at a rate of approximately 2300 pounds per hour were then introduced into the blanching tank, deposited upon the generally horizontal conveyor, and moved thereby through the blanching tank and onto the lifting conveyor by which they were removed from the blanching tank. Potato slices on the lifting conveyor were sprayed with water at a temperature of about 40° F., were blasted with compressed air to remove excess water, and then were directly admitted to a deep fat frying vat. Potato slices were continuously treated in this manner for approximately a 10 hour period of time, during which period the rate of flow of fresh hot water into the blanching tank was periodically adjusted to maintain the temperature of the blanching water therein at approximately 153° F., and the rate of withdrawal of tailings from the blanching tank was adjusted to maintain approximately constant the volume of water contained in the tank. Different varieties of potatoes were treated according to this method, and in every instance it was found that edible products having no "off" or undesirable taste were produced in the course of the deep fat frying operation.

For purposes of comparison, but not in accordance with the invention, samples of potato slices from various lots and varieties of potatoes were washed in cold water and then deep fat fried; in every such instance it was found that caramelization upon deep fat frying was substantially greater with potato slices washed in cold water as compared with potato slices treated with hot water according to the invention as set forth in the preceding paragraph.

It will be apparent that the invention is not limited to the specific embodiments described, and illustrated in the appended drawings, as various changes and modifications can be made without departing from the spirit of the claims.

This application is a continuation-in-part of application Serial No. 288,194, filed May 16, 1952, now abandoned.

Having described the invention, we claim:

1. A method for treating potato slices to prevent caramelization during subsequent deep fat frying that comprises passing potato slices through and from a treating zone containing hot blanching water at a temperature from about 150° F. to about 160° F. at a rate such that the time of contact between the slices and the blanching water is for from about 2½ minutes to about 3 minutes, admixing fresh hot water at a temperature at least about 200° F. with the blanching water at a rate sufficient to maintain the latter at a temperature within the indicated range, and withdrawing blanching water tailings from the treating zone at approximately the rate that fresh hot water is admixed with the blanching water therein.

2. A method for treating potato slices to prevent caramelization during subsequent deep fat frying that comprises passing potato slices through and from a treating zone containing hot blanching water at a temperature from about 150° F. to about 160° F. at a rate such that the time of contact between the slices and the blanching water is for from about 2 minutes to about 3½ minutes, admixing fresh hot water at a temperature at least about 200° F. with the blanching water at a rate sufficient to maintain the latter at a temperature within the indicated range, and withdrawing blanching water tailings from the treating zone at approximately the rate that fresh hot water is admixed with the blanching water therein.

3. A method for treating potato slices to prevent caramelization during subsequent deep fat frying that comprises passing potato slices through and from a treating zone containing hot blanching water at a temperature

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from about 145° F. to about 160° F., at a rate such that the time of contact between the slices and the blanching water is for from about 1½ minutes to about 8 minutes, admixing fresh hot water at a temperature substantially above 160° F. with the blanching water at a rate sufficient to maintain the latter at a temperature such that this temperature and the time of contact between the slices and the blanching water are between 1½ minutes and 4 minutes when the blanching water temperature is 160° F., between 2 minutes and 4½ minutes when the blanching water temperature is 150° F., and between 5 minutes and 8 minutes when the blanching water temperature is 145° F. and withdrawing blanching water tailings from the treating zone at approximately the rate that fresh hot water is admixed with the blanching water therein.

4. A method for treating potato slices to prevent caramelization during subsequent deep fat frying that comprises passing potato slices through and from a treating zone containing, per pound of potato slices per hour passed therethrough, at least about ⅓ gallon of hot blanching water at a temperature from about 150° F. to about 160° F., the rate of travel of slices through the treating zone being such that the time of contact between the slices and the blanching water is for from about 1½ minutes to about 4 minutes, admixing fresh water at a temperature substantially above 160° F. with the blanching water at a rate of at least about ⅓ gallon per hour per pound of potato slices per hour passed through the treating zone sufficient to maintain the latter at a temperature such that this temperature and the time of contact between the slices and the blanching water are between 1½ minutes and 4 minutes when the blanching water temperature is 160° F., between 2 minutes and 4½ minutes when the blanching water temperature is 150° F., and between 5 minutes and 8 minutes when the blanching water temperature is 145° F., and withdrawing blanching water tailings from the treating zone at approximately the rate that fresh water is admixed with the blanching water therein.

5. A method for treating potato slices to prevent caramelization during subsequent deep fat frying that comprises passing potato slices through and from a treating zone containing, per pound of potato slices per hour passed therethrough, at least about 1/10 gallon of hot blanching water at a temperature from about 145° F. to about 160° F., the rate of travel of slices through the treating zone being such that the time of contact between the slices and the blanching water is for from about 1½ minutes to about 8 minutes, admixing fresh water at a temperature of at least 160° F. with the blanching water at a rate of at least about 1/10 gallon per hour per pound of potato slices per hour passed through the treating zone sufficient to maintain the latter at a temperature such that this temperature and the time of contact between the slices and the blanching water are between 1½ and 4 minutes when the blanching water temperature is 160° F., between 2 minutes and 4½ minutes when the blanching water temperature is 150° F., and between 5 minutes and 8 minutes when the blanching water temperature is 145° F., and withdrawing blanching water tailings from the treating zone at approximately the rate that fresh water is admixed with the blanching water therein.

6. In a method for treating potato slices to prevent caramelization during subsequent deep fat frying that comprises passing potato slices through and from a treating zone containing blanching water at a predetermined temperature above room temperature, the improvement which comprises admixing fresh hot water with the blanching water at a rate sufficient to maintain the latter at the predetermined temperature, and withdrawing blanching water tailings from the treating zone at approximately the

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rate that fresh hot water is admixed with the blanching water therein.

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1,676,160   Ruffner _____ July 3, 1928
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2,418,519   McBeth _____ Apr. 8, 1947

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2,448,152   Patton _____ Aug. 31, 1948
2,498,024   Baxter _____ Feb. 21, 1950

OTHER REFERENCES

"Improvement in the Color of Potato Chips and French Fries by Certain Precooking Treatments," by T. M. Whiteman, pages 1 to 3, inclusive, reprinted from October 1951 "Potato Chipper."

FIG. 1.
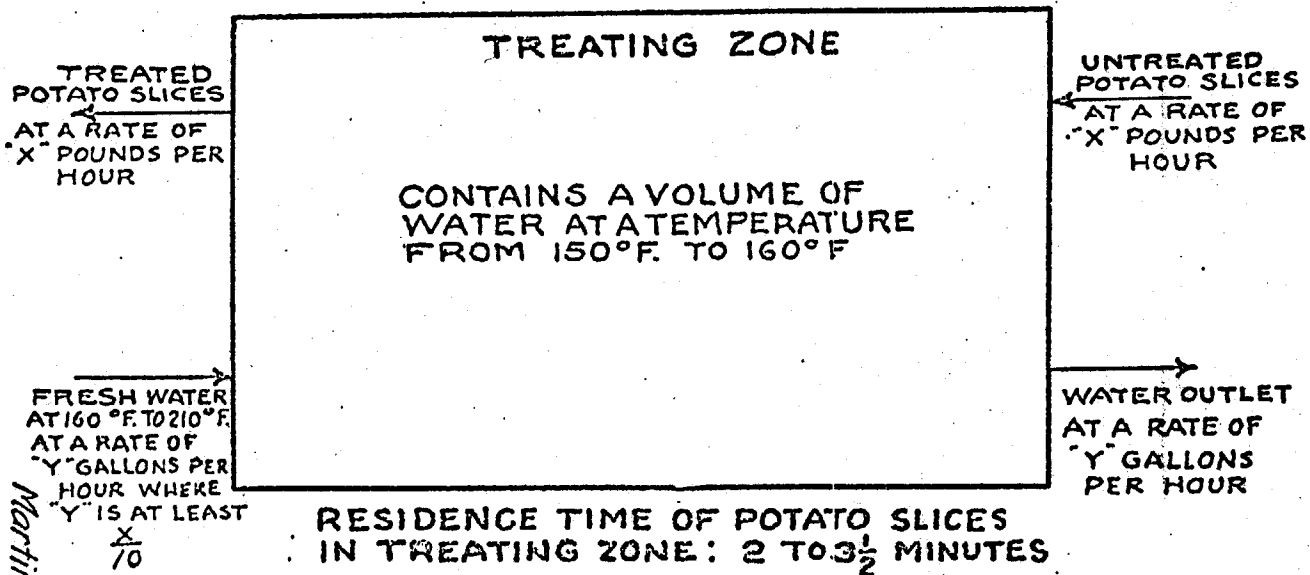
TREATED
POTATO SLICES
AT A RATE OF
"X" POUNDS PER
HOUR

UNTREATED
POTATO SLICES
AT A RATE OF
"X" POUNDS PER
HOUR

TREATING ZONE

CONTAINS A VOLUME OF
WATER AT A TEMPERATURE
FROM 150°F. TO 160°F

FRESH WATER
AT 160 °F. TO 210°F.
AT A RATE OF
"Y" GALLONS PER
HOUR WHERE
"Y" IS AT LEAST
$\frac{X}{10}$

WATER OUTLET
AT A RATE OF
"Y" GALLONS
PER HOUR

RESIDENCE TIME OF POTATO SLICES
IN TREATING ZONE: 2 TO $3\frac{1}{2}$ MINUTES

Inventors
Martin A. Janis,
Ernest G. Bremforder

By

Attorney

FIG.2

TIME OF
CONTACT
BETWEEN
POTATO
SLICES
AND
BLANCHING
WATER
IN
MINUTES

145°F.
8 MIN.

145°F
5 MIN.

150°F.
4½ MIN.

160°F.
4 MIN

150°F
2 MIN

160°F
1½ MIN.

BLANCHING WATER TEMPERATURE IN °F
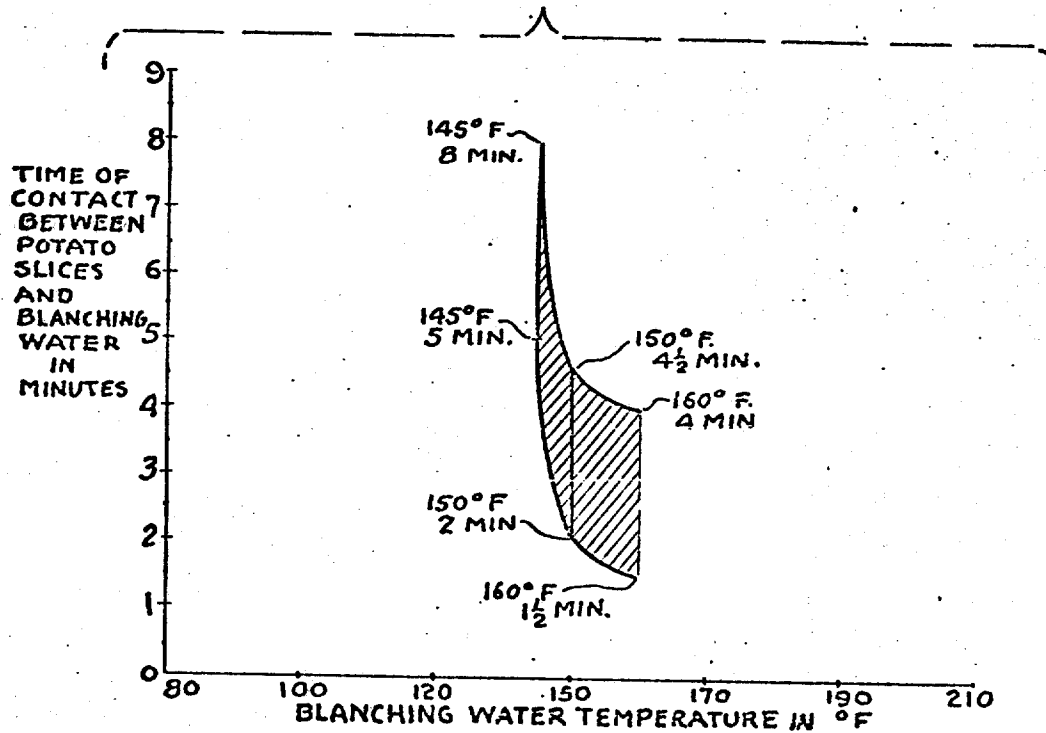
Martin A. Janis   Inventor
Ernest G. Bremsforder

By   Owen & Owen

Attorney

METHODOLOGY OF TESTING FOR CARAMEL

A. Joszt & S. Molinski

Many processes may be found in the literature concerning testing for and determination of the content of so-called sugar-color (caramel); a few are being widely applied. Though execution of said methods or reactions is not always fully specified, we shall be compiling the pertinent bibilography. (1).

Said reactions rest on the testing of diverse food samples with additives of commercial sugar-dye or caramel manufactured by the writers themselves. The latter substances however not being uniformly determined and indubitably representing mixtures of several substances, it seemed appropriate to check the methods relating thereto, particularly for caramel substances caramelane, caramelene and caramelirne, which we obtained according to the careful method of vacuum distillation of A. Pictet and N. Andrianoff (2). Though we did not succeed in obtaining by this method chemically uniform substances as was reported elsewhere (3), one must nevertheless consider the above preparations as more suitable than sugar-dye or other substances for a critical comparison of diverse methods of testing for the presence of caramel, as concerns caramel preparations obtained at normal pressure. We also felt that closer examination into the caramel reactions was appropriate, there being references in the literature that only partly characterize such methods, or not at all as concerns practical use. In LUNGE-BERL's Handbook (4), for instance, one finds, "Sugar-dye, which occasionally is used for coloring beer, so far could not be reliably tested for". H. Mastbaum's work (5) questions the applicability of A. Jaegerschmidt's method, also that of C. Amthor's for testing of sweet wines. We considered our own research in this respect the more appropriate since H. Simmich (6) assumes the presence of caramelane and caramelene according to Pictet-Gelis and of caramelirne according to Gelis (7) in commercial sugar-dyes either in the free state or in an ammonia compound.

WE wanted therefore to achieve a differentiation between these reactions concerning the above caramel components and also for the vacuum distillation products obtained during caramelisation. We therefore investigated the methods used by A. JAEGERSCHMIDT, C. AMTHOR, V. GRIESSMAYER (with L. AUBRY's modification), G. LICHTHARDT, M.N. FRADISS, C, CRAMPTON & F. SIMONS, A. STRAUB, J. NESSLER (with modification according to P. CARLES), also the reactions according to A. IHL and A. MAL-GALHAES.

EXPERIMENTS

The precise extraction process for the caramel products used by us is indicated elsewhere (8). WE shall only state here that the products were obtained from the purest refined sugar (melting point 183.3°, reducing capacity 5 mg Cu from 100 mg sugar when using the Bertrand process, 0.009% ash content) by means of vacuum heating at 187.5 - 189.5°C and 2-7 mm Hg.

For a 10% and 20% loss in weight of the above saccharose, we obtained in this fashion the caramelane and caramelene of Gelis-Pictet-Andrianov (9), such products being designated by A and B. An analogue of the Gelis carameline, designated by C, was obtained from the initial sugar with a weight loss of 25%. Besides the above caramel substances, we collected a volatile condensate in the cooled collector during that distillation proc_ess, a yellow-brown substance designated as D and characteristically smelling somewhat like benzaldehyde. Further examination into the chemical nature of this distillate has been published elsewhere (8).

Some of the properties concerning the products A-D have been listed in table 1. In part the figures in that table derive from other sources.

Table 1

Preparation	water %	ash %	organic dry subst.	melting point range	water-soluble proport. %	proportion soluble in methyl-alcohol %
A	0.564	0.08	99.36	90–110°C	100	98.64
B	0.457	0.12	99.42	120–140	98.8–99.4	44.16
C	0.600	0.18	99.22	180–220	88.6–89.35	32.00
D	27.60	0	72.40	liquid	100	100

We used further a liquid sugar-dye from the firm of Schimmel & Co from Podmokly (Czechoslovakia) in the form of liquid containing 50.0% of dry substance, which is dark and listed in the tables merely as "dye", as a control and comparison substance.

For several methods we made use of grape wine, cider and berry wine from the Rosa canina fruits. We filtered the first two through a non-heated porcelain candle; the latter was made from a fruit juice sterilized after boiling in the apparatus. The above mentioned grape wine is termed "wine" in the tables.

For comparison purposes, we made use of two malt dyes; one was prepared in Lwow at the Brauerei A.G., a caramel malt, the other was a laboratory malt made in this laboratory and dryed at 200°C.

The extinction coefficients in aqueous solutions for the caramel products A-C and for the sugar dye are listed in table 2. The most important property, the color, is thereby precisely stated. The extinction coefficients were computed by means of a Pulfrich step photometer (Zeiss), using spectral filters at the effective critical wavelengths of 434 uu to 750 uu; computations are with reference to 100 gm of dry substance.

We are most grateful to Prof. Dr. W. Lesnianski, who most kindly made this instrument available to us.

Table 2
extinction coefficients at effective critical filter
points, computed for 100 gm of dry substance

...tion	conc. %	434	463	494	530	572	619	729	750
A	0.99	87	46	31.6	16.8	10.5	6.6	2.1	2.0
B	0.99	296	207	141	85	53.4	28.2	8.5	6.1
C	0.99	77.9	44.7	30.8	18.3	11.9	8.2	2.8	2.6
C	0.25*	1000	595	365	203	110	59	13	10.8

The extinction coefficients were not determined for the D distillate
because its aqueous solution was strongly opalized and only slightly
colored.

We must stress here -- this matter being discussed at length
elsewhere (11) -- that our caramel products A-C are not to be considered
simple saccharose dehydration products but must be considered the
products of pyrogenetic reactions; this is also shown by the presence
of a volatile distillate containing carbon compounds. The A-C
products lacked precise melting points (table 1); further, products
B and C contained water-insoluble components. There is 72.4% of
dry substance in the liquid distillate D, having been condensed in
the same condenser at+7°C many times during distillation and having
been boiled off under high vacuum. This product's dry substance consists
almost entirely of furfurol compounds, mostly omega-oxymethylfurfurol
(11).

The above safeguarding conditions are not met in the caramelisation
occurring in practice or even in manufacture, notably as regards the
use of vacuum. Therefore, in the first place the pyrogenetic processes
are carried further and the decomposition products will be accumulating
more than in our caramelisation process.

With respect to commercial sugar dye, it must be stressed that usually it is obtained from the alkaline medium which, - in view of the acid properties of the caramelisation products as also shown by us-, renders likely H. Simmich's assumed compounds of caramelane, caramelene and carameline, for instance with ammonia (12). Another consequence from the alkali effect is in the color deepening of the caramel substances, which we already observed for caramel colloids (13).

When the coefficients of extinction are being compared in table 2, we will notice that the solution of the deepest dyed preparation B is much weaker in color than the sugar dye. The extinction coefficients of preparations A-C are very similar in behavior to that of the sugar dye; our preparations merely show a lesser extinction for the short-wave light, i.e., uv. Further, the extinction coefficients for preparations A and C are nearly the same, that is, the color of their aqueous solutions is the same. The deepest colored preparation is B which is about three times as strongly colored as preparations A and C. It appears the 1% distillate solutions for D and our preparations A and C contained some wine dye; product B and the sugar dye already contained this dye in 0.1% concentration.

1. A. JAEGERSCHMIDT's METHOD

a) USING RESORCIN. According to this procedure, 100 cc of solution are cleared by means of 2 cc aqueous egg white solution (1꜆)and, under constant agitation, will be heated to complete egg white coagulation (15-70 minutes); the solution then is concentrated in the water bath to syrup consistency and the ethyl ether extract from this syrup is boiled off in porcelain dishes and treated with two drops,for each, of resorcin/hydrochloric acid solution (1 gm of resorcin in 100 cc of concentrated hydrochloric acid). A red color then is supposed to indicate presence of caramel.

The results obtained by such a method are listed in table 3.

b) WITH ACETONE. The syrup obtained under a) is extracted, not with ether, but with acetone, and if necessary, the extract will be filtered and made to react with an equal volume of concentrated sulfuric acid; the presence of caramel is supposedly indicated by violet-red coloring.

We carried out the above method not only qualitatively, but also quantitatively, by always filling to 10 cc, by means of acetone, the acetone extracts, and made to react each 5 cc of the above solution with 5 cc of hydrochloric acid, and we made colorimetric comparisons. Qualitative results are shown in table 4.

TABLE 3

Preparation	concentrations			
	1%	0.1%	0.01%	0.001%
A	+++	++	+	0
B	+++	++	+	0
C	++	0	0	0
D	++++	++++	+++	+
sugar dye	+++	+++	+	0
saccharose (control)	0	0	0	0

Table 3 shows colorings as follows: ++++: intense red; +++: red; ++: pink; + faintly pink; 0: colorless.

TABLE 4

A	++	0	0	0
B	++	0	0	0
C	+	0	0	0
D	+++	++	0	0
sugar dye	++	++	0	0
saccharose (control)	0	0	0	0

Table 4 shows the colors as follows: +++: strong red-violet; ++: red-violet; +: weak red violet; 0: colorless.

Quantitative determinations show that if we take 100 as the reference for the color intensity of 0.1% solution from D distillate, then One in 1% solution of preparation A will be 15, for B it will be 12 and for sugar dye it will be 86.

It is generally known that Jaegerschmidt's method is based on one kind of the so-called Fiehe reaction (14). The omega-oxymethylfurfurol according to Ekenstein and Blanksma (15) provides the basis for such reaction. It is obvious therefore that our D distillate, the dry substance of which most likely consists to the greater part of omega-oxymethylfurfurol, will provide a positive reaction for the resorcin reaction when still at 0.001% concentration. For preparations A, B and C the reaction is increasingly weaker in that order; preparation C for instance provides a positive reaction only when in 1% concentration. The sugar dye can still be shown to be present when in 0.01% solution. It is likely therefore that all these products contain small amounts of furfurol derivatives. In this respect one must also stress that 0.001 - 1% saccharose solutions reacted with egg white and heated for 70 minutes in identical manner with other samples, will show no trace of omega-oxymethylfurfurol.

H. Mastbaum (16) objects as regards this method that several wines not colored with caramel nevertheless provide a positive reaction under the Jaegerschmidt method. Actually Kruisher etal (17) have shown the presence of oxymethylfurfurol in several wines not artificially colored. One must take into account however that when a ketose such as fructose is subjected to high temperatures or even to an organic acid, there is the possibility that oxymethylfurfurol will be generated. In view of the sensitivity of this reaction, errors easily may arise.

Comparing the two executions of this method, we will notice the larger sensitivity of the former one, namely the resorcin reaction. While there still is a positive reaction from the distillate at 0.1% solution when using the acetone method, --table 4 --, there no longer is a reaction at 0.01%. In comparison with the resorcin sample, the sensitivity of the distillate and of products A and B is therefore 1/100; for sugar dye, it is 1/10.

C. AMTHOR's METHOD

10 cc of the solution to be analyzed are reacted with 30-50 cc
paraldehyde and an equal amount of water-free alcohol and are agitated
so that good mixing occurs. If caramel is present, a brown, smeary
precipitate strongly adhering to the glass is supposed to be formed
after 24 hours. The precipitate is decanted with absolute alcohol
and dissolved in hot water and reacted with 1 gm of phenylhydrazine
hydrochloride and 2 gm of sodium acetate. In order to remove any
resinous by-products, the liquid is coated with ether. If the original
sample held caramel, a red or dirty brown flocculent precipitate,
soluble in soda lye and ammonium but insoluble in hydrochloric acid
will now be formed in the cold aqueous solution. In this method
as in Jaegerschmidt's, it must be emphasized that concentrating
of the solutions to be tested never may take place at higher tempera-
tures but only under vacuum at room temperature.

The results obtained are listed in table 5.

As seen from table 5, preparation B is distinctive among the
caramel preparations in that it still may show proof at a 0.1% concen-
tration, much as the sugar dye. The amount of precipitates obtained
at this concentration however is so minute that we had to give up
further testing with phenyl hydrazine hydrochloride. The precipitate
obtained from the sugar dye in a larger amount produced an ample
precipitate with phenyl hydrazin at low temperature; on the other hand,
the paraldehyde precipitates obtained for products A, B and C with
phenyl hydrazine hydrochloride cause clouding only after some time
and when heated; an insignificant amount of precipitate was formed
after 24 hours, which was of a red-brownish color.

Table 5

preparation	precipitates in solutions for concentrations of			
	5%	1%	0.1%	0.01%
A	++	++	0	0
B	++	++	+	0
C	++	++	0	0
D	++	0	0	0
sugar dye	++	++	+ (traces)	0

++ in table 5 indicates a precipitate, while + indicates a very small one.

The nature of this reaction is unknown; however the paraldehyde precipitates must have a different composition when reacting with sugar dye than when they react with our caramel preparations, as may be concluded from their different behaviors with respect to phenyl hydrazine hydrochloride and sodium acetate.

Amthor sees the reaction as the "formation of caramel hydrazone". No statement may be made with respect to sugar dye, still one was entitled to surmise with respect to our A,B, and C preparations that the phenyl hydrazine precipitation possibly occurs because of impurities such as monoses contained in these products or caused by hydrolysis. This is also rendered plausible because of the microscopic appearance of these precipitates where, besides red spherical shapes, we could also observe the yellow needles characteristic of the monoses' osazone.

As regards the paraldehyde precipitate, obtained from the 5% distillate, we must observe it was entirely different from the other precipitates; it was a yellow powder that did not react with phenyl hydrazine. Amthor too mentions the possibility of the occurrence of paraldehyde precipitates which do not react with phenyl hydrazine.

3. GRIESSMAYER's METHOD as altered by L. AUBRY.

A sample of the liquid to be tested is reacted with ammonium sulfate to saturation and thereafter with an equal volume of alcohol (96%), and agitated. After some time the lower ammonium sulfate layer separates from the upper one of alcohol. If there was caramel salt or sugar dye in the liquid, then one of the layers will be colored by the caramel malt color, namely the lower layer, while the upper one of alcohol will be dyed brown by the sugar dye. This method is supposed to be particularly useful when wishing to discriminate between dyeings from caramel salt and sugar dye, since the former is more easily soluble in sulfate solution and the latter in alcohol. Table 6 shows the results.

Table 6
Coloring of liquid layers for initial-solution concentrations of:

preparation	1%			0.1%	
	upper layer	lower layer	ratio: upper/lower layers	upper layer	lower layer
A	yell.	yell.	1.7/1	light yell.	colorless
B	"	"	2.5/1	"	"
C	"	colorless	–	colorless	"
D	"	"	–	"	"
lab. malt	"	brown	1/4.4	–	–
Brewery malt	"	"	1/21.1	colorless	yellow
sugar dye	deep brown	light yell.	220/1	yellow	colorless

We indicated the results colorimetrically by means of a Universalcolorimeter Leitz in the form of the color intensity ratio of the upper layer of alcohol to the lower sulfate-saturated layer. The malt dryed by us in the laboratory served as a control for classifying the brewery malt, but not for controlling quantitatively its color.

As may be seen from table 6, there is a large difference between the solubility of commercial sugar dye for the two solvents used: the alcohol layer was dyed 220 times more intensively than the saturated sulfate layer.

For preparations A,B and C, this difference is much smaller.The baked malts however show an opposite form of their color solubility.

It should be observed here as well that after addition of ammonium sulfate, the solutions of products A, B and C became appreciably lighter. This may be explained by circumstances explained by us elsewhere (18): the caramel colloids contained in our preparations are subject to the coagulating effect of the ammonium sulfate, and the colloidal, salting-out part of those products does form their strongest dyed component.

4. LICHTHARDT's METHOD.

5 cc of the liquid to be tested are reacted with 5 cc of clear, diluted with sulfuric-acid tannin solution (1gm of tannin, 30 cc water, 0.75 gm sulfuric acid and water to 50 gm). In case of clouding following the mixing of the solutions, the liquid is carefully heated in order to obtain clearing and then let , stand for 12-24 hours.

If caramel is present, precipitation of a brown color occurs. In order to obtain quantitative information, we collected the precipitate after 24 hours on hardened, dried filters and always washed in the same manner with diluted sulfuric acid; weighing took place after drying and at $50^{o}C$ in vacuum.

Table 7 shows the results.

This procedure is meant for testing for caramel in spices, vinegar and alcoholic liquids.

AS shown from table 7, the sensitivity of this procedure varies much for our preparations and sugar dye. The latter causes precipitation of 15 mg in 0.1% and 5 cc solution. Such an amount is not reached even for 5% concentration for the strongest reacting product B. It is unquestionable that the compounds (which we do not yet know) reacting here occur in much lesser concentrations in our preparations than they do in sugar dye. There are also quantitative differences between the preparations A, B and C with respect to the amount of precipitates, which decrease in this order: product B, distillate, product C and preparation A. Saccharose control is negative.

Table 7
mg of precipitates from 5 cc of solution
for concentrations of

preparation	5%	1%	0.1%	0.01%
A	1-2	1-2	0	0
B	6-7	3	0	0
C	3-4	2	0	0
D	3-4	2	0	0
sugar dye	-	20	15	0
saccharose (control)	0	0	0	0

5. FRADISS' METHOD

The substance to be tested is boiled for two hours at the reflux condenser with methyl alcohol in the dry state (sic), then it is washed with methyl alcohol and the solution is partially reacted with amyl alcohol till precipitation ceases. This procedure is repeated twice or three times; the amyl alcohol precipitates are purified, filtered, and dried at 90°C and weighed.

Table 8 shows the results.

Actually the Fradiss method was meant only for testing for caramel in sugar manufacturing processes. We stress that the solubility of our preparations in methyl alcohol decreases in the following order:

D distillate (100% solubility); preparations A, B, C, with 68% of insoluble components for latter. ProductA is the easiest to precipitate with amyl alcohol; the others, including sugar dye, are but slightly precipitated, and this circumstance much decreases the value of the method.

Table 8

preparation	soluble in methyl alcohol %	precipitate of amyl alcohol (%)
A	98.6	28.5
B	44.1	10.5
C	32.0	5.0
D	100.0	1.5
sugar dye	90.7	13.5

6. METHOD I, CRAMPTON & SIMONS

50 cc of the solution to be tested are reacted with 25 gm of fuller's earth, agitated and left to stand for $\frac{1}{2}$ hour. In contrast to the natural colors, the sugar dye will be adsorbed. When sugar dye is present the solution will therefore be discolored.

Since we did not have the fuller earth of Crampton and Simons, we attempted using the earths current in petroleum refining, namely floridine and tonsile (3 gm and 25 gm to 100 cc solution - table 9--) also fuller earth 441 (25 gm to each of the 50 cc of solutions being tested -- table 10). In the latter case we carried out quantitative determinations by means of the Leitz Universalcolorimeter.

We investigated not only the aqueous solutions of our own products, but also a rye brandy aged in an oak cask and termed "Starka", further also a natural wine without artificial coloring.

As can be seen, tonsile discolors more than floridine. Thus, 3% doses discolored 0.1% solutions, and 25% doses discolored 1% solutions of our preparations A, B and C. A negative result obtains for Starka, which contains a natural dye from the oak cask.

Table 10 shows there is strong adsorption of fuller earth for all caramel products (A-C) and also for sugar dye. The rye brandy substance is adsorbed much less, the natural wine not at all. In view of these results and also because of the ease of execution, this method seems quite remarkable; it does however require closer and further testing concerning any adsorption of other natural dyes and the determination of the optimal amount and quality of the adsorption earth.

Table 9

Preparation	concentration	tonsil earth 3%	earth 25%	floridine earth 3%	earth 25%
A	1.0	0	+++	0	0
	0.1	++	+++	++	+++
B	1.0	0	+++	0	+++
	o.1	+++	+++	++	+++
C	1.0	0	+++	0	+++
	0.1	+++	+++	++	+++
D	1.0	0	++	0	+++
	0.1	+++	+++	++	+++
sugar dye	1.0	–	++	–	++
	0.1	+++	+++	+	++
Starka	–	0	0	0	0
Wine	–	++	–	++	–

For table 9, the symbols mean, +++ : complete discoloration;
++: partial discoloration; +: very weak discoloration; 0: no discolor.

Table 10

Preparation	concentration	discoloration effect with fuller earth in % (colorimetric measurements)
A	1.0	88.8
B	1.0	100.0
	0.1	100.0
C	1.0	100.0
D	1.0	100.0
sugar dye	1.0	85.4
	0.1	94.6
Starka	–	38.5
Wine	–	0

To this must be added a preliminarily observed selectivity of the adsorption earths used as regards the adsorptions of different dyes contained in our preparations and in our rye brandy. It appears that in this partial adsorption, various dyes also were adsorbed varyingly, so that certain color differences were caused in the fields of view of the colorimeter.

7. METHOD II, CRAMPTON & SIMONS

The remainder of the 50 cc of liquid to be tested and that was boiled off in a water bath is reacted with 25 cc of absolute ethyl alcohol in a 50 cc flask (graduated) and is filled with water up to the index line. 25 cc of this solution together with 50 cc of ethyl ether are shaken in a special separating funnel with a narrow part and a 25 cc line at its middle (19). The lower layer after half an hour is filled up with water to the 25 cc line, is shaken again, and then colorimetrically examined for comparison with the solution to be tested.

If color intensity has been decreased by less than 36.4% by this extraction, a synthetic sugar must be present in the solution according to Crampton and Simons. The relevant results are listed in table 11.

Table 11 shows that for all caramel products tested, including sugar dye, there is correctness as to the fundamentals of the method and the results obtained. However the rye brandy Starka, that assuredly was free of synthetic coloring, indicates weaker coloring by 34% after the ether extraction above. The entire method therefore must be checked further in order to determine the adsorptions limits with a vaster amount of research material.

Table 11

Preparation	discoloring effect with ether in % (colorimetric)
A	0
B	0
C	0
D	6
sugar dye	1
Starka	27

8. STRAUB'S PROCESS

10 cc of the solution to be tested are diluted to clear wine color and after addition of 3 cc of 1% stannous chloride solution and of 0.5 gm of potassium acetate are heated till there is flocculent precipitation. If caramel is present, this precipitate is yellow or brown.

We tested the solutions of our preparations according to this method and in one of concentrations corresponding to the above color (A and C and the distillate D =1%, B and the sugar dye =0.1%).

The precipitates obtained showed

1. for grape wine (from juice filtered through a Chamberland candle): white coloring.

2. for cider (treated as in 1): light yellow coloring

3. Wine from Rosa canina (juice was hot, sterilized after boiling): yellow precipitate coloring

This qualitative method therefore is well suited for all our caramel products, also for the sugar dye. The grape wine tested under 1 also is determined as being natural rather than synthetic; but the cider (2) behaves differently: the precipitate is light yellow. Therefore a wine from rosa canina (3) even produces a yellow precipitate. As is seen, further investigation must determine which the natural substances are that are carried along by the precipitation in the Straub reaction, or are adsorbed. This reaction seems unsuited for caramel testing in fruit wines.

9. NESSLER's METHOD AS MODIFIED BY P. CARLES

a) brandy testing: the brandy is made to react by 1/6 of its volume with fresh egg-clear; after agitation, a brandy dyed with sugar dye will not change, but for natural coloring, there is supposedly a partial lightening.

b) wine test: Fresh eggwhite filtered through flannel is mixed with the same volume of water; 2 cc of this liquid are shaken together with 20 cc of the wine to be tested, and filtered. There is no discoloration for the wine colored with sugar dye, but natural dyes will precipitate.

Our Starka was treated in that fashion. 20 cc were reacted with 2-4 cc of egg white and filtered after shaking. The color of the solution ranged from brown into orange. The color of the 1% sugar dye solution for the same treatment was also partially altered, but much less.

The above method is recommended in the Lunge-Berl handbook; none of our products of caramelisation (A.B. and C)were discolored, at most there was some fading, whereas the wines performed as expected from the Straub method, or nearly so. The grape wine with cold-sterilized juice was wholly discolored ; the cider with cold-sterilized juice showed only weak discoloration; the berry wine from rosa canina, which was sterilized hot, was not discolored at all.

With respect to this method we may state therefore that comparison of rye brandy reaction with 1% sugar dye did indeed confirm the applicability of this method, even though the differences in discoloration are not particularly striking.

However with respect to the method for wine testing, conclusions similar to those applying to Straub's method must be drawn here too.

10. IHL's REACTIONS

In particular the caramel obtained from saccharose is supposed to yield red compounds with resorcin or with hydrochloride-like solutions of pyrogallol acid. Ihl provided no particular details concerning his methods, but for the case of positive results, his reaction might become the basis for pertinent methods.

As already previously mentioned, resorcin is the fundamental reagent in the Jaegerschmidt method, which was discussed above. We carried out as follows the pyrogallol acid reaction: a knife's tip of pyrogallol acid (about 0.05 -o.1 gm) was dissolved in 2 cc of the liquid to be tested and the liquid was made to react with an equal volume of concentrated hydrochloric acid. If caramel is present, a deep red precipitate is expected (table 12).

Thus IHL's reaction will only be observed for the distillate and is likely to be the reaction of the furfurol compounds. When comparing table 12 with table 3, one will however notice the appreciably higher sensitivity of Jaegerschmidt's method.

Table 12   Pyrogallol acid and HCl reaction in
                  solutions of concentrations of

preparations	1%	0.1%
A	no precipitation;	solution becomes brown.
B	clouding; solution browns	neither precipitation nor color changes
C	clouding; solution browns	neither precipitation nor color changes
D	red precipitate; solution turns deep red	no precipitation; solution turns pink
sugar dye	no precipitation; solution browns	neither precipitation nor color changes

11. MAGALHES' REACTIONS

a) 100 cc of the solution to be tested together with 10 cc of 10% potassium sulfate solution and a bit of cotton are boiled. If caramel is present, the cotton is expected to turn light orange; this color should be fast even after water and ammonium washing.

b) 20 cc of the liquid to be tested together with 10 cc of lead acetate (5%) are shaken, filtered and then mixed with amyl alcohol and shaken again. According to Magalhaes, the amyl alcohol layer may then be colored yellow.

The results we obtained are as follows:

a) 1% solutions of products A, B and C turned the cotton light yellow for reaction a); the cotton in a 1% solution of product D assumed a lght brown color and in 0.1% solution an intensely yellow color fast to water and ammonium. The sugar dye solution for a 1.0% concentration colored the cotton light orange (the dyeing intensity nearly corresponds to that of the distillate at 0.1%). For a sugar dye concentration of 0.1% the result was entirely insignificant: only a hardly visible yellow dyeing of the cotton occurred.

b) No amyl alcohol coloring is observed for the 1% solutions of products A, B and C when reacting according to b) of Magalhaes' method; however this solvent turns light yellow when reacting with the 1% solution of the distillate. The sugar dye sample shows no coloring.

c) for method c, there are negative results for products A, B and C and the sugar dye; that is, the amyl alcohol remained uncolored. Only a 1% distillate solution showed a yellow coloring in the alcohol layer.

The result occurring under a) and with respect to the distillate is worthy of emphasis. This product causes the largest cotton coloring and thereby is different from all other products tested. This affinity is also evidenced with respect to human skin. Besides the distillate, there is also a positive result for the same sample a) for a 1% sugar dye solution; the other solutions only lead to negative results.

Similarly, a positive result may only be noted for reactions b) and c) with respect to the 1% distillate. The other preparations - including the sugar dye -, all led to negative results; one must therefore conclude that in the Magalhaes reactions, the reacting substances are not the colored caramel products, but rather a by-product of the decomposition associated with caramelisation -- an analogue of the distillate being tested.

Because of the compilation of the results concerning testing methods for caramel as performed above, we reach the final conclusion that good results may only be achieved by using Jaegerschmidt's method (particularly so with resorcin), next by the methods of Amthor and Crampton/Simons (by means of discoloration earths), as well as that of Griessmayer/Aubry (beer testing).

The Jaegerschmidt method, which to us seems the most proper, however must be qualified by the need for another controlling one; the reacting substance in this instance, namely oxymethylfurfurol, is a decomposition product of sugar and therefore does not necessarily originate from the sugar dye being used as a coloring agent (20), but may also originate in the natural component of the solution being tested, as due from heating or from acidic effects, and it might for instance be formed from fructose. Therefore, for the Jaegerschmidt method as well as for that of Amthor, boiling-off of the solution being investigated may only take place at room temperature and preferably under vacuum.

The Lichthardt method provided good results as regards the sugar dye; however all the results concerning our caramel preparations were insignificant. One cannot for the time being determine, accordingly, which compound is the reacting substance.

Closer testing is required for the methods of Straub, Nessler/Carles, also Crampton/Simons (ether extraction), particularly with respect to the natural wine and brandy dyes.

The Fradiss method was shown non-quantitative and of little sensitivity, and thus insufficient in practice for testing in general for caramel.

The applicability of the Ihl and Magahaes methods or reactions for testing for caramel seems dubious. The surprising factor in this respect is the yellow to orange coloring of the cotton, showing that such may be also achieved with caramel, and not only with tar substances.

SUMMARY

1. The testing methods for caramel as developed by A. Jaeger-schmidt, C. Amthor, V. Griessmayer, L. Aubry, G. Lichthardt, M. Fradiss, C. Crampton/F. Simons, A. Straub, J. Nessler/P.Carles, and the caramel reactions of A. Ihl and A. Magalhaes were closely examined; the comparison substances used by us were the Pictet/Andrianoff saccharose caramelisation products obtained by a modified vacuum method, the modification being ours.

2. The coloring of the caramel products, namely caramelane (preparation A) and caramelene (preparation B) of Pictet/Andrianoff and carameline (preparation C) of A. Gelis, also that of commercial sugar dye, are determined by their extinction coefficients.

3. The most sensitive method for detecting caramel has been Jaegerschmidt's when using ethyl ether extracts and resorcin; the use of acetone was found to provide less sensitivity. It appears that the reacting substance in both methods is omega-oxymethylfurfurol, which is a by-product of caramelisation. Therefore the reaction is strongest for the volatile distillation product D of saccharose. Sugar dye, preparations A, B and C cause the reaction in that descending order.

4. The reacting substances in the less sensitive method of C. Amthor are colored caramel substances, but not the volatile decomposition products. The sensitivity of this method is highest for product B (caramelene 0.1%) and for sugar dye (0.1%).

5. The method of Griessmayer/Aubry was made quantitative by means of a colorimeter. For caramel malt and sugar dye, satisfactory results were obtained.

6. For commercial sugar dye, the Lichthardt method is far more sensitive than it is for our caramel products and for the volatile caramelisation product D.

7. The Fradiss method applied to our caramel products and also to sugar dye leads to wholly unsatisfactory results.

8. The Crampton/Simons method for discoloring caramel solutions leads to complete discoloration for products A, B and C and also for the sugar dye, for certain dosages of adsorption earths such as tonsil, floridin and fuller's earth.

9. Testing by means of the Crampton/Simons method and with ether extracts shows that none of our caramelisation products may be extracted with ether. The natural color of an aged rye brandy may be extracted to 27%.

10. Straub's method resulted in the formation of colored precipitates for all caramel preparations tested, also for a few wine species.

11. When using the Carles method, the caramel products were not discolored with respect to egg white. Only the aged rye brandy and a few wines were fairly well discolored.

12. The reactions cited by Ihl are based on a method similar to Jaegerschmidt's; the latter's however allows showing appreciably smaller concentrations of omega-oxymethylfurfurol in solution.

13. The Magalhaes reactions were caused by volatile caramelisation products: therefore they may only show the presence of those distilled products in the solution.

Untersuchungen über Caramelnachweismethoden.

Von

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Als Nachweis- und Bestimmungsmethoden des Gehaltes der Lebensmittel an sog.
Zuckercouleur (Zuckerfarbe, Caramel) werden in der Literatur viele Verfahren ange-
geben, von denen einige sogar breitere Anwendung gefunden haben. Obwohl die Aus-
führung der genannten Methoden oder Reaktionen nicht immer genau präzisiert ist,
stellen wir doch die darauf zielende Literatur[1]) zusammen.

Die genannten Reaktionen beruhen auf der Prüfung verschiedener Lebensmittel-
proben mit Zusätzen von käuflicher Zuckerfarbe oder von Caramel, welches von den
Verfassern selbst hergestellt wurde. Da aber letztere Substanzen nicht einheitlich bestimmt
sind und sicher Mischungen mehrerer Stoffe darstellen, schien es uns zweckmäßig, die
diesbezüglichen Methoden nachzuprüfen, und zwar auf Grund der Caramelsubstanzen
Caramelan, Caramelen und Caramelin, die wir nach der schonenden Vakuumdestillations-
methode von A. Pictet und N. Andrianoff[2]) erhalten haben. Es ist uns zwar nicht
gelungen, auf Grund dieser Methode chemisch einheitliche Stoffe zu bekommen — wie
von anderer Seite[3], angegeben wurde —, doch müssen wir obige Präparate für eine
kritische und vergleichende Untersuchung verschiedener Caramelnachweismethoden als
mehr geeignet ansehen als z. B. die Zuckercouleur oder andere, bei normalem Druck
gewonnene Caramelpräparate. Nähere Untersuchungen der Caramelreaktionen schienen
uns auch zweckmäßig, da wir in der Literatur Notizen antreffen, die diese Methoden
als unvollständig oder gar nicht verwendbar charakterisieren. Im Handbuch von Lunge-
Berl[4]) finden wir z. B. die Worte: „Die Zuckerfarbe, welche zum Färben des Bieres
mitunter verwendet wird, ist durch keine Methode bisher sicher nachzuweisen.“

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[4]) G. Lunge-E. Berl: Chem. techn. Untersuchungsmethoden, 7. Aufl., 4, 381 (1921).

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Die Arbeit von H. Mastbaum[1] bezweifelt wieder die Anwendbarkeit der Methode von A. Jägerschmidt sowie der von C. Amthor für die Untersuchung der Süßweine. Unsere diesbezüglichen Untersuchungen hielten wir um so mehr für zweckmäßig, weil H. Simmich[2] das Vorhandensein von Caramelan und Caramelen von Pictet-Gélis und von Caramelin von Gélis[3] in käuflichen Zuckerfarben im freien Zustande oder in Verbindung mit Ammoniak annimmt.

In der vorliegenden Arbeit bezweckten wir also, eine Differenzierung dieser Reaktionen zwischen den obigen Teilsubstanzen des Caramels sowie dem bei der Caramelisierung erhaltenen Vakuumdestillationsprodukt vorzunehmen. Es wurden von uns die Methoden von A. Jägerschmidt, C. Amthor, V. Griessmayer mit der Modifikation nach L. Aubry, G. Lichthardt, M. N. Fradiss, C. Crampton und F. Simons, A. Straub, J. Neßler mit der Modifikation nach P. Carles, sowie die Reaktionen von A. Ihl und A. Magalhaès der Prüfung unterzogen.

Experimenteller Teil.

Die genaue Gewinnungsmethode der von uns benutzten Caramelprodukte wurde an anderer Stelle[4] angegeben. Hier sei nur bemerkt, daß die Produkte aus reinster Raffinade (Schmelzpunkt 183,8°, Reduktionsfähigkeit 5 mg Cu aus 100 mg Zucker bei Anwendung der Bertrand-Methode, 0,009% Aschegehalt) durch Erwärmung im Vakuum bei 187,5—189,5° und 2—7 mm Quecksilberdruck gewonnen wurden.

Beim 10%- und 20%-igen Gewichtsverlust obiger Saccharose erhielten wir auf diese Weise die dem Caramelan und Caramelen von Gélis-Pictet-Andrianoff[3] entsprechenden, weiter mit A und B bezeichneten Produkte. Ein Analogon des Caramelins von Gélis[3] — mit C bezeichnet — wurde ebenso bei 25% Gewichtsverlust des Ausgangszuckers gewonnen. Außer obigen Caramelsubstanzen haben wir während dieser Vakuumdestillationen in der gekühlten Vorlage ein flüchtiges Kondensat gesammelt, und zwar eine gelblich-braune, charakteristisch — etwa nach Benzaldehyd — riechende Flüssigkeit, die weiter mit D bezeichnet wird. Unsere näheren Untersuchungen der chemischen Natur dieses Destillates wurden an anderer Stelle[4] veröffentlicht.

In der Tabelle 1 wurden einige, die angewendeten Produkte A—D betreffende Einzelheiten zusammengestellt. Teilweise entstammen die Zahlen der Tabelle 1 unserer anderweitigen Veröffentlichungen[6].

Tabelle 1.

Prä-parat	Wasser %	Asche %	Organische Trocken-substanz %	Schmelz-punkt-grenzen	Wasser-löslicher Anteil %	Methylalkohol-löslicher Anteil %
A	0,564	0,08	99,36	90°—110°	100	98,64
B	0,457	0,12	99,42	120°—140°	98,8—99,4	44,16
C	0,600	0,18	99,22	180°—220°	88,6—89,35	32,00
D	27,60	0	72,40	flüssig	100	100

[1] Chem.-Ztg. 1933, 57, 959; Diese Zeitschrift 1933, 66, 254.

[2] Zeitschr. Ver. Deutsch. Zuckerind. 1926, 76, 1.

[3] A. Gélis: Ann. de chim. 1858, (3) 52, 356.

[4] A. Joszt und S. Moliński: Biochem. Zeitschr. 1935, 282, 269.

[5] Helv. Chim. Acta 1924, 7, 703.

[6] A. Joszt und S. Moliński: Biochem. Zeitschr. 1935, 282, 269 und Kolloidchem. Beihefte 1935, 42, 357.

Als Vergleichs- und Kontrollsubstanz benutzten wir außerdem eine flüssige Zucker-couleur der Firma Schimmel & Co. in Podmoklý (Tschechoslowakei) in der Form einer dunklen, 50.0% Trockensubstanz enthaltenden Flüssigkeit, die in den Tabellen kurz „Couleur" bezeichnet wird.

Dabei haben wir auch bei einigen Methoden vergleichsweise einen Trauben- und Äpfelwein sowie auch einen Beerenwein aus den Früchten der Rosa canina angewandt. Die beiden ersten Weine wurden von uns selbst aus einem nichterhitzten, durch eine Porzellankerze filtrierten, der letzte aber aus einem im Apparat nach Koch steri-lisierten Most hergestellt. Der obengenannte Traubenwein wird in den Tabellen kurz als „Wein" bezeichnet.

Vergleichsweise wurden von uns auch zwei Farbmalze verwendet; es waren dies ein in der Brauerei A.G. in Lwów hergestelltes Caramelmalz und ein Laboratoriums-malz, das im hiesigen Laboratorium dargestellt und bei 200° gedarrt worden war.

Für die angewendeten Caramelprodukte A—C und für die Zuckercouleur geben wir in der Tabelle 2 die Extinktionskoeffizienten ihrer wässerigen Lösungen an. Da-durch wird ihre wichtigste Eigenschaft — die Farbe — genau präzisiert. Die Ex-tinktionskoeffizienten wurden mittels eines Stufenphotometers von Pulfrich (Firma Zeiss) bei Anwendung der Spektralfilter bei den wirksamen Filterschwerpunkten von 434 bis 750 $\mu\mu$ bestimmt und auf 100 g Trockensubstanz berechnet.

Wir sprechen hier Herrn Prof. Dr. W. Leśniański, welcher uns gütigst obigen Apparat zur Verfügung gestellt hatte, unseren verbindlichsten Dank aus.

Tabelle 2.

Präparat	Konzen-tration %	Extinktionskoeffizienten bei wirksamem Filterschwerpunkt in $\mu\mu$, auf 100 g Trockensubstanz berechnet:							
		434	463	494	530	572	619	729	750
A	0,99	87	46	31,6	16,8	10,5	6.6	2,1	2,0
B	0,99	296	207	141	85	53,4	28,2	8,5	6,1
C	0,99	77,9	44,7	30,8	18,3	11,9	8,2	2,8	2,6
D	0,25[1]	1000	595	365	203	110	59	13	10,8

Die Extinktionskoeffizienten wurden für das Destillat D nicht bestimmt, da seine Wasserlösung stark opalisiert und nur leicht gefärbt ist.

Wir müssen hier nachdrücklich betonen — dieser Umstand wird an anderer Stelle[2] ausführlicher besprochen —, daß unsere Caramelstoffe A—C nicht als einfache Saccharoseentwässerungsprodukte, sondern als Produkte pyrogenetischer Reaktionen an-gesehen werden müssen; dies wird auch durch die Existenz eines flüchtigen, Kohlen-stoffverbindungen enthaltenden Destillates bewiesen. Die Produkte A—C hatten keine genau präzisierten Schmelzpunkten (Tabelle 1); außerdem enthielten die Präparate B und C wasserunlösliche Anteile. Das flüssige Destillat D enthält 72,4% Trockensubstanz, da es während vieler Destillationen in derselben auf +7° gekühlten Vorlage kondensiert und im hohen Vakuum durch Abdampfen des Wassers konzentriert wurde. Die Trocken-substanz dieses Produktes besteht fast ausschließlich aus Furfurolverbindungen, vor allem aus ω-Oxymethylfurfurol[2].

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[1] Die Konzentration der Zuckercouleur D wurde in der Tab. 2 und in den weiteren Tabellen in g der Couleurflüssigkeit auf ccm der Lösung angegeben.

[2] A. Joszt und S. Moliński: Biochem. Zeitschr. 1935, 282, 269.

Bei der in der Praxis vorkommenden oder sogar fabrikationsmäßig ausgeführten Caramelisierung treffen wir die obigen schonenden Bedingungen, insbesondere die Anwendung von Vakuum überhaupt nicht. Infolgedessen werden im ersten Falle die pyrogenetischen Prozesse weiter geführt und die Zersetzungsprodukte stärker als bei unserem Caramelisationsprozesse angehäuft.

Was die Zuckerfarbe des Handels anbelangt, so muß hervorgehoben werden, daß man sie gewöhnlich im alkalischen Medium gewinnt, was — angesichts des auch von uns bewiesenen sauren Charakters der Caramelisationsprodukte — die Entstehung der von H. Simmich[1] angenommenen Caramelan-, Caramelen- und Caramelinverbindungen, z. B. mit Ammoniak, wahrscheinlich macht. Eine weitere Folge der Alkaliwirkung besteht in einer Farbenvertiefung der Caramelstoffe, was wir auch schon bei den Caramelkolloiden[2] bemerkt haben.

Beim Vergleich der Extinktionskoeffizienten (Tab. 2) bemerken wir zunächst, daß die Lösung unseres am stärksten gefärbten Präparates B vielmal schwächer als die Zuckercouleur gefärbt ist. Die Extinktionskurven der Präparate A—C verlaufen aber ähnlich wie bei der Couleurkurve, nur weisen unsere Präparate eine schwächere Extinktion des kurzwelligen, d. h. des violetten Lichtes auf. Weiter haben die Präparate A und C fast identische Extinktionskoeffizienten, d. h. eine gleiche Farbe ihrer Wasserlösungen. Am stärksten, und war etwa dreimal so stark gefärbt wie die Präparate A und C, erscheint das Präparat B. Augenscheinlich hatten die 1%-igen Lösungen unseres Destillates D sowie die unserer Präparate A und C eine Weinfarbe; dieselbe Farbe aber hatte das Produkt B und die Zuckercouleur schon in 0,1%-iger Konzentration.

1. Methode nach A. Jägerschmidt.

a) Mit Resorcin. Nach dieser Methode werden 100 ccm Lösung mit 2 ccm wässeriger Eiweißlösung (1:1) geklärt und beim fortwährenden Rühren bis zur vollständigen Eiweißkoagulation erwärmt (15—70 Minuten); sodann wird die Lösung im Wasserbade zur Sirupdicke konzentriert, der Äthylätherextrakt dieses Sirupes in Porzellanschälchen abgedampft und mit je 2 Tropfen von Resorcin-Salzsäure-Lösung (1 g Resorcin in 100 ccm konzentrierter Salzsäure) versetzt. Eine Rotfärbung soll dabei die Anwesenheit des Caramels beweisen.

Die bei der Anwendung obiger Methode gewonnenen Ergebnisse sind in der Tabelle 3 dargestellt.

Tabelle 3.

Präparat	Konzentration			
	1%	0,1%	0,01%	0,001%
A	+---	++-	+	0
B	-+--	++-	-+	0
C	-+-	0	0	0
D	++++	++++	+++	+
Couleur	+++-	--+	+	0
Saccharose	0	0	0	0
(Kontrolle)				

In Tabelle 3 sind die Färbungen, wie folgr. bezeichnet: +++- = intensiv rot, +++ = rot, ++ = rosafarbig, + = schwach rosafarbig, 0 = farblos.

b) Mit Aceton. Der nach a) erhaltene Sirup wird nicht mit Äther, sondern mit Aceton extrahiert, der Extrakt nötigenfalls filtriert und mit dem gleichen Volumen von konz. Salzsäure versetzt: violett-rote Färbung soll die Anwesenheit des Caramels andeuten.

[1] Zeitschr. Ver. Deutsch. Zuckerind. 1926, 76, 1.
[2] A. Joszt und S. Moliński: Kolloidchem. Beihefte 1935, 42, 367.

Wir haben aber obige Methode nicht nur qualitativ, sondern auch quantitativ durchgeführt, indem wir die Acetonextrakte immer auf 10 ccm mit Aceton auffüllten, je 5 ccm obiger Lösung mit 5 ccm Salzsäure versetzten und colorimetrisch verglichen. Die qualitativen Ergebnisse geben wir in der Tabelle 4 an.

Quantitative Bestimmungen ergaben, daß, wenn wir die Intensität der Färbung mit 0,1%-iger Lösung von Destillat D mit 100 bezeichnen, dieselbe in 1%-iger Lösung von Präparat A durch die Zahl 15, von B durch 12 und von der Zuckercouleur durch 86 ausgedrückt werden kann.

Es ist allgemein bekannt, daß die Methode nach Jägerschmidt auf einer Form der sog. Reaktion von Fiehe[1] basiert. Das

. Tabelle 4.

Präparat	Konzentration			
	1%	0,1%	0,01%	0,001%
A	++	0	0	0
B	++	0	0	0
C	+	0	0	0
D	+++	++	0	0
Couleur	++	+	0	0
Saccharose (Kontrolle)	0	0	0	0

In Tabelle 4 sind die Färbungen, wie folgt, bezeichnet: +++ = stark rotviolett, ++ = rotviolett, + = schwach rotviolett, 0 = farblos.

ω-Oxymethylfurfurol bildet nämlich nach W. A. van Ekenstein und J. Blanksma[2] die Grundlage obiger Reaktion. Es ist daher selbstverständlich, daß unser Destillat D, dessen Trockensubstanz höchstwahrscheinlich hauptsächlich aus ω-Oxymethylfurfurol besteht, bei der Resorcin-Reaktion noch in 0,001%-iger Konzentration eine positive Reaktion gibt. Bei den Präparaten A, B und C ist diese Reaktion der Reihe nach immer schwächer: Präparat C gibt z. B. eine positive Reaktion erst in 1%-iger Konzentration. Die Zuckercouleur kann noch in 0,01%-iger Lösung nachgewiesen werden. Wahrscheinlich also enthalten alle diese Produkte kleine Mengen des obigen Furfurolderivates. Es muß dazu auch hervorgehoben werden, daß 0,001—1%-ige Saccharoselösungen, mit Eiweiß versetzt und 70 Minuten lang mit anderen Proben identisch erhitzt, keine Spur von ω-Oxymethylfurfurol aufweisen.

Gegen diese Methode wird von H. Mastbaum[3] eingewendet, daß einige nicht mit Caramel gefärbte Weine nach der Jägerschmidt-Methode untersucht doch eine positive Reaktion ergeben. In der Tat haben C. I. Kruisheer, N. J. M. Vorstman und L. C. E. Kniphorst[4] in einigen, nicht künstlich gefärbten Weinen die Anwesenheit von Oxymethylfurfurol bewiesen. Man muß also immer beachten, daß, wenn eine Ketose, z. B. Fructose, der Einwirkung einer höheren Temperatur oder sogar einer organischen Säure ausgesetzt wird, die Möglichkeit der Entstehung von Oxymethylfurfurol besteht; wegen der Empfindlichkeit dieser Reaktion aber kann dies leicht zu irrtümlichen Ergebnissen führen.

Beim Vergleich der beiden Ausführungsformen dieser Methode stellen wir eine viel größere Empfindlichkeit der ersteren, nämlich der Resorcinreaktion fest. Bei der Acetonmethode gibt unser Destillat eine positive Reaktion noch in 0,1%-iger Lösung (Tabelle 4), bei 0,01% aber bleibt schon die Reaktion aus. Im Vergleich mit der Resorcinprobe ist also die Empfindlichkeit bei dem Destillat sowie bei den Produkten A und B 100-mal so gering und bei der Zuckercouleur ungefähr 10-mal so gering.

[1] E. Raumer, Diese Zeitschrift 1909, 17, 115 und 126.
[2] Ber. Deutsch. Chem. Ges. 1910, 43, 2355.
[3] Chem.-Ztg. 1933, 57, 959; Diese Zeitschrift 1933, 66, 254.
[4] Diese Zeitschrift 1935, 69, 570.

2. Methode nach C. Amthor.

10 ccm der zu analysierenden Lösung werden mit 30—50 ccm Paraldehyd und mit soviel wasserfreiem Alkohol versetzt und geschüttelt, daß sich die Flüssigkeiten gut mischen. Bei Anwesenheit von Caramel soll dabei nach 24 Stunden ein brauner, schmieriger, stark am Glase anhaftender Niederschlag gebildet werden. Dieser wird mit absol. Alkohol dekantiert, in heißem Wasser gelöst und mit 1 g Phenylhydrazin-hydrochlorid und 2 g Natriumacetat versetzt. Zur Entfernung etwaiger harziger Neben-produkte wird weiter die Flüssigkeit mit Äther überschichtet. Wenn die ursprüngliche Probe Caramel enthält, wird jetzt in der kalten wässerigen Lösung ein rot- bis schmutzigbrauner, flockiger, in Ammoniak und Natronlauge löslicher, in salzsaurer Lösung aber unlöslicher Niederschlag gebildet. Es muß aber bei dieser Methode und ebenso bei der Methode nach Jägerschmidt nachdrücklich hervorgehoben werden, daß eine Konzentrierung der zu untersuchenden Lösungen nie bei einer höheren Temperatur, sondern nur im Vakuum bei Zimmertemperatur stattfinden darf.

Die gewonnenen Ergebnisse sind in der Tabelle 5 angegeben.

Wie aus der Tabelle 5 ersichtlich ist, zeichnet sich unter unseren Caramelprodukten das Präparat B dadurch aus, daß man es noch in 0.1%-iger Konzentration ähnlich wie die Couleur nachweisen kann. Die bei dieser Konzen-tration erhaltenen Niederschlagsmengen waren aber so klein, daß wir auf eine weitere Unter-suchung mit Phenylhydrazinhydrochlorid ver-

Tabelle 5.

Prä-parat	Niederschlag in Lösungen bei der Konzentration			
	5%	1%	0,1%	0,01%
A	−÷	÷÷	0	0
B	−÷	÷÷	÷	0
C	−÷	÷÷	0	0
D	−−	0	0	0
Couleur	÷−	÷÷	÷ (Spuren)	0

In der Tabelle 5 wird mit ÷÷ ein Niederschlag und mit ÷ ein sehr geringer Niederschlag bezeichnet.

zichten mußten. Der aus Zuckerfarbe bei höherer Konzentration in größeren Mengen gewonnene Niederschlag gab mit Phenylhydrazin in der Kälte sogleich einen reich-lichen Niederschlag; dagegen ergaben die in den Produkten A, B und C erhaltenen Paraldehydniederschläge mit Phenylhydrazinhydrochlorid erst nach einiger Zeit und nach Erwärmung eine Trübung: nach 24 Stunden fiel hier eine unbedeutende Menge eines rötlich-braunen Niederschlages aus.

Das Wesen dieser Reaktion ist bisher unbekannt, die Paraldehydniederschläge müssen aber bei der Zuckercouleur einerseits und unseren Caramelpräparaten anderer-seits eine verschiedene Zusammensetzung haben, was man aus ihrem unterschiedlichen Verhalten gegen Phenylhydrazinhydrochlorid und Natriumacetat folgern kann.

Die Reaktion wird von Amthor als eine „Caramelhydrazonbildung" aufgefaßt. Bezüglich der Couleur kann hier vorläufig nichts Näheres gesagt werden, aber bei unseren Präparaten A, B und C kann man doch vermuten, daß der Phenylhydrazin-niederschlag aus möglicherweise in diesen Produkten enthaltenen oder durch Hydrolyse gebildeten Verunreinigungen, z. B. Monosen, entsteht. Dies wird auch durch das mikroskopische Aussehen dieser Niederschläge wahrscheinlich gemacht, in denen wir neben rötlichen kugelförmigen Gebilden die für Osazone der Monosen charakteristischen gelben Nadeln beobachtet haben.

Zu dem aus 5%-iger Destillat-Lösung gewonnenen Paraldehydniederschlag müssen wir bemerken, daß er von den anderen Niederschlägen ganz verschieden war, und

.war ein gelbes, mit Phenylhydrazin nicht reagierendes Pulver darstellte. Auch von Amthor wird in seiner Arbeit die Möglichkeit der Entstehung solcher mit Phenylhydrazin nicht reagierender Paraldehydniederschläge erwähnt.

3. Methode nach V. Grießmayer in der Abänderung von L. Aubry.

Eine Probe der zu untersuchenden Flüssigkeit wird bis zur Sättigung mit festem Ammoniumsulfat und nachher mit dem gleichen Volumen Alkohol (96%) versetzt und geschüttelt. Nach einiger Zeit teilt sich die untere Ammoniumsulfatschicht von der oberen alkoholischen ab. Wenn die untersuchte Lösung mit Caramelmalz oder mit Zuckercouleur gefärbt war, so erscheint jetzt eine Färbung einer der Schichten, und zwar wird durch Caramelmalzfarbe die untere Schicht, durch die Zuckerfarbe aber die obere Alkoholschicht braun gefärbt. Diese Methode soll besonders zur Unterscheidung einer Färbung mit Caramelmalz von der mit Zuckerfarbe dienen, denn die erstere ist leichter in der Sulfatlösung und die zweite im Alkohol löslich. Die Ergebnisse werden in der Tabelle 6 dargestellt.

Tabelle 6.

Präparat	Die Färbung der Flüssigkeitsschichten bei der Konzentration der Anfangslösung				
	1%			0,1%	
	Obere Schicht	Untere Schicht	Relativ: Obere Schicht Untere Schicht	Obere Schicht	Untere Schicht
A	gelb	gelb	$\frac{1,7}{1}$	hellgelb	farblos
B	gelb	gelb	$\frac{2,5}{1}$	hellgelb	farblos
C	gelb	farblos	—	farblos	farblos
D	gelb	farblos	—	farblos	farblos
Laboratoriumsmalz	gelb	braun	$\frac{1}{4,4}$	--	—
Brauereimalz	gelb	braun	$\frac{1}{21,1}$	farblos	gelb
Couleur	dunkelbraun	hellgelb	$\frac{220}{1}$	gelb	farblos

Die Ergebnisse haben wir colorimetrisch (Universalcolorimeter Leitz) quantitativ als Verhältnis der Farbintensität der oberen Alkoholschicht zu der unteren mit Sulfat gesättigten Schicht angegeben. Das von uns im Laboratorium gedarrte Malz diente als Kontrolle der Gattung des Brauereimalzes, nicht aber zur quantitativen Kontrolle seiner Färbung.

Wie aus der Tabelle 6 zu ersehen ist, besteht ein sehr großer Unterschied in der Löslichkeit der käuflichen Zuckerfarbe in den beiden angewandten Lösungsmitteln: die Alkoholschicht war 220-mal so intensiv gefärbt wie die gesättigte Sulfatschicht.

Bei den Präparaten A, B, C ist diese Differenz viel kleiner. Die gebrannten Malze dagegen weisen eine entgegengesetzte Gestaltung ihrer Farbenlöslichkeit auf.

Es soll hier auch bemerkt werden, daß die Lösungen der Produkte A, B und C nach Zugabe von Ammoniumsulfat bedeutend heller wurden. Dies läßt sich durch

einen von uns an einer anderen Stelle[1]) festgestellten Umstand erklären: Die in unseren Präparaten enthaltenen Caramelkolloide unterliegen der koagulierenden Wirkung von Ammonsulfat, und der aussalzbare kolloidale Teil dieser Produkte bildet doch eben ihren am stärksten gefärbten Bestandteil.

4. Methode nach G. Lichthardt.

5 ccm der zu untersuchenden Flüssigkeit werden mit 5 ccm einer klaren, verdünnten, schwefelsauren Tanninlösung versetzt (1 g Tannin, 30 ccm Wasser, 0,75 g konz. Schwefelsäure und Wasser bis 50 g). Im Falle einer Trübung nach der Vermischung der Lösungen wird die Flüssigkeit noch durch vorsichtiges Erwärmen geklärt und dann 12—24 Stunden stehen gelassen. Nach Verlauf dieser Zeit fällt bei Anwesenheit von Caramel ein brauner Niederschlag aus. Zur zahlenmäßigen Orientierung haben wir nach 24 Stunden die Niederschläge auf gehärteten, trockenen Filtern gesammelt, immer auf dieselbe Weise mit verdünnter Schwefelsäure gewaschen und nach dem Trocknen (bei 50° im Vakuum) gewogen.

Tabelle 7 enthält die Ergebnisse.

Diese Methode war für den Nachweis von Caramel in Gewürzen, Essig und alkoholischen Flüssigkeiten bestimmt.

Wie aus der Tabelle 7 ersichtlich, ist die Empfindlichkeit dieser Methode bei unseren Präparaten und der Couleur sehr verschieden. Die Zuckerfarbe gibt nämlich in 0,1%-iger Lösung 15 mg Niederschlag aus 5 ccm Lösung.

Tabelle 7.

Präparat	mg Niederschlag aus 5 ccm der Lösung von der Konzentration			
	5 %	1 %	0.1 %	0.01 %
A	1—2	1—2	0	0
B	6—7	3	0	0
C	3—4	2	0	0
D	3—4	2	0	0
Couleur	—	20	15	0
Saccharose (Kontrolle)	0	0	0	0

Diese Niederschlagsmenge wird sogar bei 5%-iger Konzentration des am stärksten reagierenden Produktes B nicht erreicht. Es ist also zweifellos, daß die hier reagierenden, uns noch unbekannten Verbindungen in unseren Präparaten in viel geringerer Konzentration als in der Zuckercouleur auftreten. Auch sehen wir zwischen den Präparaten A, B und C quantitative Unterschiede, und zwar vermindert sich die Menge des Niederschlages in der Reihenfolge: Produkt B, Destillat, Produkt C und Präparat A. Die Kontrolle mit Saccharose fällt dabei negativ aus.

5. Methode nach M. Fradiss.

Die zu untersuchende Substanz wird im trockenen Zustande 2 Stunden am Rückflußkühler mit Methylalkohol gekocht, dann wird filtriert, mit Methylalkohol gewaschen und die Lösung portionsweise bis zum Aufhören der Niederschlagsbildung mit Amylalkohol versetzt. Dies wird zwei- bis dreimal wiederholt; die Amylalkoholniederschläge werden vereinigt, filtriert, bei 90° getrocknet und gewogen.

Die Ergebnisse sind in der Tabelle 8 angegeben.

Die Fradiss-Methode war eigentlich nur für den Nachweis des Caramels in den Zucker-

Tabelle 8.

Präparat	Methylalkohollöslich %	Amylalkoholniederschlag %
A	98,6	28,5
B	44,1	10,5
C	32,0	5,0
D	100,0	1,5
Couleur	90,7	13,5

[1]) Kolloidchem. Beihefte 1935, 42, 367.

fabrikationsprodukten bestimmt. Wir müssen bemerken, daß die Löslichkeit unserer Präparate im Methylalkohol in folgender Reihe abnimmt: Destillat D (100% Löslichkeit), Präparat A, dann B und endlich Präparat C (68% unlösliche Bestandteile). Mit Amylalkohol wird weiter das Produkt A am leichtesten, die übrigen aber — einschließlich der Couleur — nur schwach ausgefällt, was den Wert dieser Methode stark herabsetzt.

6. Methode I nach C. Crampton und F. Simons.

50 ccm der zu untersuchenden Lösung werden mit 25 g Walkererde versetzt, geschüttelt und ¹/₂ Stunde stehen gelassen. Die Zuckerfarbe wird dabei im Gegensatz zu den Naturfarbstoffen adsorbiert. Bei Anwesenheit von Couleur wird also die Lösung entfärbt.

Da wir über die von Crampton und Simons benutzte Walkererde nicht verfügten, versuchten wir die Anwendung der in den Petroleumraffinerien verwendeten Erden und zwar Floridin und Tonsil (3 g und 25 g auf 100 ccm Lösung (Tab. 9), sowie Fullererde Nr. 411 (25 g auf je 50 ccm der zu untersuchenden Lösung (Tab. 10). Im letzteren Falle haben wir quantitative Bestimmungen mit dem Universalcolorimeter von Leitz ausgeführt.

Mit dieser Methode wurden von uns nicht nur die Wasserlösungen unserer Produkte, sondern auch ein im Eichenfaß gealterter, weiter kurz als „Starka" bezeichneter Roggenbranntwein und auch ein ohne künstliche Färbung hergestellter Traubenwein untersucht.

Wie ersichtlich, hat Tonsil eine stärker entfärbende Wirkung als Floridin. Und zwar entfärbt eine 3%-ige Gabe 0.1%-ige Lösungen und eine 25%-ige Gabe 1%-ige Lösungen unserer Präparate A, B und C. Ähnlich wird auch die käufliche Zuckercouleur absorbiert. Ein negatives Resultat ergibt die „Starka", welche einen natürlichen, vom Eichenfaß herstammenden Farbstoff enthält.

In der Tabelle 10 tritt ein sehr starkes Adsorptionsvermögen der Fullererde für alle Caramelprodukte (A—C) sowie für die Couleur auf. Der Roggenbranntweinfarbstoff wird schon viel weniger, der natürliche Traubenweinfarbstoff aber gar nicht absorbiert. Angesichts dieser Ergebnisse sowie wegen ihrer einfachen Ausführung scheint uns diese Methode bemerkenswert zu sein: sie verlangt aber noch eine nähere Unter-

Tabelle 9.

Prä-parat	Konzen-tration %	Entfärbungseffekt mit			
		Tonsilerde		Floridin	
		3%	25%	3%	25%
A	1,0	0	+++	0	0
	0,1	++	+++	++	+++
B	1,0	0	+++	0	+++
	0,1	+++	+++	++	++
C	1,0	0	+++	0	+++
	0,1	+++	+++	++	+++
D	1,0	0	++	0	+++
	0,1	+++	+++	++	+++
Couleur	1,0	—	++	—	++
	0,1	+++	+++	+	++
„Starka"	—	0	0	0	0
Wein	—	++	—	++	—

In der Tabelle 9 gelten folgende Zeichen: +++ = gänzliche Entfärbung, ++ = partielle Entfärbung, + = sehr schwache Entfärbung, 0 = ohne Entfärbung.

Tabelle 10.

Präparat	Konzen-tration %	Entfärbungs-effekt mit Fullererde in % (colorimetrisch bestimmt)
A	1,0	88,8
B	1,0	100,0
	0,1	100,0
C	1,0	100,0
D	1,0	100,0
Couleur	1,0	85,4
	0,1	94,6
„Starka"	—	38,5
Wein	—	0

suchung über die etwaige Adsorption noch anderer Naturfarbstoffe, sowie die Feststellung optimaler Menge und Qualität der Adsorptionserde. Es kommt noch dazu eine von uns vorläufig beobachtete Selektivität der benutzten Adsorptionserden in der Adsorption verschiedener in unseren Präparaten und im Roggenbranntwein enthaltenen Farbstoffe. Augenscheinlich waren bei diesen Teiladsorptionen verschiedene Farbsubstanzen auch verschieden absorbiert, was dann gewisse Farbenunterschiede der im Colorimeter verglichenen Sichtfelder hervorrief.

7. Methode II nach C. Crampton und F. Simons.

Der Rückstand von 50 ccm der im Wasserbade eingedampften zu untersuchenden Flüssigkeit wird in einem 50 ccm-Meßkolben mit 25 ccm absol. Äthylalkohol versetzt und mit Wasser zur Marke aufgefüllt. 25 ccm dieser Lösung werden mit 50 ccm Äthyläther in einem besonderen Scheidetrichter[1] mit Verengung und einer 25 ccm-Marke in der Mitte ausgeschüttelt. Die untere Schicht wird nach einer halben Stunde zur 25 ccm Marke mit Wasser ergänzt, wieder ausgeschüttelt und im Vergleich mit der zu untersuchenden Lösung colorimetrisch untersucht.

Wenn durch diese Extraktion die Farbenintensität um weniger als 36.4% erniedrigt wird, muß sich nach Crampton und Simons in der Lösung ein künstlicher Zuckerfarbstoff vorfinden. Die diesbezüglichen Ergebnisse sind in der Tabelle 11 angegeben.

Die Tabelle 11 zeigt, daß bei allen untersuchten Caramelprodukten, einschließlich der Couleur, die Grundlage der Methode und die gewonnenen Ergebnisse richtig sind. Der Roggenbranntwein „Starka" dagegen, der ganz sicher frei von künstlicher Färbung war, zeigt nach obiger Ätherextraktion eine um 27% schwächere Färbung. Es muß also die ganze Methode noch weiter untersucht werden, um auf einem umfangreicheren Forschungsmaterial die Adsorptionsgrenzen feststellen zu können.

Tabelle 11.

Präparat	Entfärbungseffekt mit Äther in % (colorimetrisch)
A	0
B	0
C	0
D	6
Couleur	1
„Starka"	27

8. Methode nach A. Straub.

10 ccm der zu untersuchenden Lösung werden bis zur hellen Weinfarbe verdünnt und nach Zusatz von 3 ccm Zinnchlorürlösung (1%) und 0.5 g Kaliumacetat bis zur Abscheidung eines flockigen Niederschlages erwärmt. Bei Anwesenheit von Caramel ist dieser Niederschlag gelb oder braun gefärbt.

Mit dieser Methode haben wir die Lösungen unserer Präparate in einer der obigen Farbe entsprechenden Konzentration (A und C sowie das Destillat D = 1%, B und die Couleur = 0.1%) untersucht.

Die erhaltenen Niederschläge zeigten beim:

1. Traubenwein (aus einem durch Chamberlandkerze filtrierten Most): weiße Färbung;

2. Apfelwein (der Most wie bei 1. behandelt): hellgelbe Färbung;

3. Wein aus Rosa canina (der Most heiß, im Apparat nach Koch sterilisiert): gelbe Niederschlagsfärbung.

Diese qualitative Methode ist daher für alle unsere Caramelprodukte, sowie für die Couleur gut geeignet. Auch der unter 1. untersuchte Traubenwein wird durch diese

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[1] C. A. Crampton und F. D. Simons: Journ. Amer. Chem. Soc. 1900, 22, 810.

thode richtig als nicht künstlich gefärbt qualifiziert: der Apfelwein (2) dagegen verhält sich anders: die Fällung ist hier hellgelb. Desgleichen gibt ein Wein aus Rosa canina 3. sogar einen gelben Niederschlag. Wie man sieht, muß also erst durch weitere Untersuchungen festgestellt werden, welche Naturfarbstoffe bei der Straub-Reaktion durch den Niederschlag mitgerissen bezw. adsorbiert werden. Für den Caramelnachweis in Obstweinen scheint diese Reaktion ungeeignet zu sein.

9. Methode nach J. Neßler in der Abänderung nach P. Carles.

a) Branntweinuntersuchung: Der Branntwein wird mit $\frac{1}{6}$ seines Volumens mit frischem Eierklar versetzt; nach dem Schütteln wird ein mit Couleur gefärbter Branntwein nicht verändert, bei einer natürlichen Färbung aber soll eine teilweise Aufhellung erfolgen.

b) Weinuntersuchung: Frisches, durch Flanell filtriertes Eiereiweiß wird mit dem gleichen Volumen Wasser vermischt; 2 ccm dieser Flüssigkeit werden mit 20 ccm des zu untersuchenden Weines geschüttelt und filtriert. Bei dem mit Zuckerfarbe gefärbten Wein darf keine Entfärbung stattfinden, dagegen wird der natürliche Farbstoff des Weines gefällt.

Auf diese Weise wurde unser Roggenbranntwein „Starka" behandelt. 20 ccm davon wurden mit 3—4 ccm Eiereiweiß versetzt und nach dem Schütteln filtriert. Die Farbe der Lösung ging von Braun in Orange über. Die Farbe der 1%-igen Zucker-couleurlösung wurde bei derselben Behandlung auch teilweise, aber viel weniger verändert.

Bei der obigen, im Handbuch von Lunge-Berl für die Untersuchung des Weines empfohlenen Methode wurde keines von unseren Caramelisationsprodukten (A, B und C) entfärbt, höchstens hat sich dabei ein Erblassen herausgestellt, während die Weine sich analog wie bei der Straub-Methode verhielten. Der Traubenwein (Most kalt sterilisiert) nämlich wurde gänzlich entfärbt, während der Apfelwein (kalt sterilisierter Most) eine schwache Entfärbung zeigte: der Beerenwein aus Rosa canina (heiß sterilisiert) wurde dagegen gar nicht entfärbt.

Bezüglich dieser Methode können wir also behaupten, daß der Vergleich der Reaktion mit Roggenbranntwein und mit 1%-iger Couleur die Anwendbarkeit der Methode bestätigt, obwohl die Unterschiede der Entfärbung nicht besonders auffallend sind.

Was aber die Anwendung der Methode zur Weinuntersuchung betrifft, so müssen hier analoge Folgerungen wie vorher bei der Methode nach Straub gezogen werden.

10. Reaktionen nach A. Ihl.

Insbesondere das aus Saccharose gewonnene Caramel soll mit Resorcin oder mit salzsaurer Pyrogallussäurelösung rote Verbindungen geben. Ihl hat keine besondere Untersuchungsmethode angegeben, doch könnte seine Reaktion im Falle positiver Resultate als Grundlage entsprechender Methoden aufgefaßt werden.

Das Resorcin ist — wie wir früher erwähnt haben — das Grundreagens der Jägerschmidt-Methode, mit welcher wir uns schon oben befaßt haben; die Reaktion mit Pyrogallussäure haben wir auf folgende Weise durchgeführt: In etwa 2 ccm der zu untersuchenden Lösung wurde eine Messerspitze (etwa 0.05—0.1 g) von Pyrogallussäure gelöst und die Flüssigkeit mit dem gleichen Volumen konz. Salzsäure versetzt. Bei Anwesenheit von Caramel soll eine dunkelrote Fällung hervortreten (Tab. 12).

Die von Ihl angegebene Reaktion wird also nur beim Destillat beobachtet, muß also wahrscheinlich als Reaktion der Furfurolverbindungen angesehen werden. Beim Vergleich der Tab. 12 mit der Tab. 3 tritt aber deutlich die viel höhere Empfindlichkeit der Methode von Jägerschmidt hervor.

Tabelle 12.

Präparat	Reaktion mit Pyrogallussäure und HCl in Lösungen von der Konzentration	
	1%	0,1%
A	keine Fällung; die Lösung wird gebräunt	
B	Trübung; die Lösung wird gebräunt	keine Fällung und Farbänderung
C	Trübung; die Lösung wird gebräunt	keine Fällung und Farbänderung
D	roter Niederschlag; die Lösung wird intensiv rot	keine Fällung; die Lösung wird rosafarbig
Couleur	keine Fällung; die Lösung wird gebräunt	keine Fällung und Farbänderung

11. Reaktionen nach A. Magalhaês.

a) 100 ccm der zu untersuchenden Lösung werden 10 Minuten mit 10 ccm Kaliumsulfatlösung (10%) und mit einem Stückchen Wolle gekocht. Die Wolle soll dabei im Falle der Anwesenheit von Caramel hellorange gefärbt werden; auch soll sie diese Farbe nach dem Waschen mit Wasser und Ammoniak nicht verlieren.

b) 20 ccm der zu untersuchenden Flüssigkeit werden mit 10 ccm Bleiacetatlösung (5%) geschüttelt, filtriert und dann mit Amylalkohol vermischt und wieder geschüttelt. Bei Anwesenheit vom Caramel soll in der Amylalkoholschicht eine orangegelbe Färbung auftreten.

c) 100 ccm einer das Caramel enthaltenden Flüssigkeitsprobe werden mit Ammoniak gesättigt und mit Amylalkohol geschüttelt. Nach Magalhaês darf dabei die Amylalkoholschicht gelborange gefärbt werden.

Magalhaês warnt aber hier vor Verwechslungen und irrtümlichen Schlußfolgerungen, da alle drei Reaktionen sowohl durch Teerfarbstoffe, als auch durch Caramel hervorgerufen werden können.

Die von uns gewonnenen Ergebnisse waren die folgenden:

a) 1%-ige Lösungen der Produkte A, B und C färbten bei der Reaktion a die Wolle hellgelb; in 1%-iger Lösung des Produktes D nahm die Wolle eine hellbraune und in 0,1%-iger eine intensiv gelbe wasser- und ammoniakechte Färbung an. Die Lösung der Couleur in einer Konzentration von 1,0% färbte die Wolle hell-orange (die Intensität der Färbung entspricht beinahe der Färbung, welche beim 0,1%-igen Destillate auftritt). Bei der Zuckercouleurkonzentration von 0,1% war das Resultat ganz unbedeutend; es wurde eine nur schwer sichtbare gelbe Färbung der Wolle erhalten.

b) 1%-ige Lösungen der Produkte A, B und C geben bei der Reaktion b nach Magalhaês keine Färbung des Amylalkohols, dagegen färbt sich dieses Lösungsmittel hellgelb bei der Reaktion mit 1%-iger Lösung des Destillates. Bei der Probe mit Couleur ist keine Färbung sichtbar.

c) 1%-ige Lösungen der Produkte A, B und C und der Couleur liefern bei der Methode c ein negatives Ergebnis; der Amylalkohol blieb also ungefärbt; nur eine 1%-ige Destillatlösung wies in der Alkoholschicht eine gelbe Färbung auf.

Aus diesen Versuchen ist besonders das bei a mit dem Destillat auftretende Ergebnis hervorzuheben. Dieses Produkt veranlaßt die stärkste Wollefärbung und unterscheidet sich dadurch von allen anderen untersuchten Produkten. Dieselbe Färbungs-

fänigkeit zeigt es auch der Menschenhaut gegenüber. Außer dem Destillat kann noch aber ein positives Resultat derselben Probe a bei 1%-iger Couleurlösung gesprochen werden: andere Lösungen lieferten nur negative Ergebnisse.

In ähnlicher Weise kann ein positives Ergebnis bei den Reaktionen b und c nur beim 1%-igen Destillat festgestellt werden. Die übrigen Präparate, einschließlich der Couleur, haben alle ein negatives Resultat gegeben. Es muß also gefolgert werden, daß bei den Magalhaès-Reaktionen nicht die gefärbten Caramelprodukte, sondern ein Nebenprodukt der mit der Caramelisation verbundenen Zersetzung — ein Analogon des untersuchten Destillates — als reagierende Substanz hervortritt.

Bei der Zusammenstellung der Ergebnisse obiger Caramelnachweismethoden kommen wir zur Schlußfolgerung, daß gute Ergebnisse nur durch die Anwendung der Jägerschmidt-Methode (besonders mit Resorcin), dann der Amthor- und der Crampton- und Simons-Methode (mittels Entfärbungserden), sowie auch nach der Grießmayer- und Aubry-Methode (bei der Bierprüfung) erreicht werden.

Bei der Jägerschmidt-Methode, welche uns die richtigste zu sein scheint, muß aber der Vorbehalt gemacht werden, daß ihre Ergebnisse noch mit einer anderen Methode kontrolliert werden müssen; die hier reagierende Substanz, nämlich das ω-Oxymethylfurfurol ist ein Zersetzungsprodukt des Zuckers, braucht also nicht nur der als Färbungsmittel benutzten Zuckercouleur zu entstammen[1]), sondern kann auch unter Temperatureinwirkung oder bei Säureeinfluß aus einem natürlichen Bestandteil der untersuchten Lösung, und zwar der Fructose, gebildet werden. Daher ist sowohl bei der Jägerschmidt- als auch bei der Amthor-Methode ein Eindampfen der zu untersuchenden Lösung nur bei Zimmertemperatur, am besten im Vakuum zulässig.

Mit der Lichthardt-Methode wurden für Zuckercouleur ebenfalls gute Ergebnisse gewonnen: bei allen unseren Caramelpräparaten aber waren die Ergebnisse nur unbedeutend; es kann also vorläufig nicht entschieden werden, welche Verbindung die reagierende Substanz dieser Methode darstellt.

Für die Methoden nach Straub, Neßler und Carles, sowie Crampton und Simons (Ätherextraktion) sind noch nähere Untersuchungen nötig, und zwar in bezug auf die natürlichen Wein- und Branntweinfarben.

Die Fradiss-Methode hat sich zur praktischen Anwendung als nicht quantitativ, wenig empfindlich, also für den allgemeinen Caramelnachweis als unzureichend erwiesen.

Die Anwendbarkeit der Ihl- und Magalhaès-Reaktionen als Caramelnachweismethoden scheint bedenklich zu sein. Auffallend dagegen ist die hier auftretende Gelb- bis Orangefärbung der Wolle, welche also nicht nur mit Teerfarbstoffen, sondern auch mit Caramel zu erreichen ist.

Zusammenfassung.

1. Die Caramelnachweismethoden von A. Jägerschmidt, C. Amthor, V. Grießmayer, L. Aubry, G. Lichthardt, M. Fradiss, C. Crampton und F. Simons, A. Straub, J. Neßler und P. Carles sowie die Caramelreaktionen von A. Ihl und A. Magalhaès wurden näher geprüft: als Vergleichssubstanzen wurden die nach der von uns abgeänderten Vakummethode der Saccharosecaramelisation von A. Pictet und N. Andrianoff gewonnenen Produkte benutzt.

[1]) Vergl. Kruisheer u. a.: Diese Zeitschrift 1935, 69, 570.

2. Es wurde die Farbung der Caramelprodukte, welche dem Caramelan Praparat A und Caramelen Präparat B von A. Pictet und N. Andrjanoff und dem Caramelin Praparat C von A. Gélis entsprechen, sowie die Farbung der käuflichen Zuckercouleur mittels ihrer Extinktions-koeffizienten bestimmt.

3. Die Methode von A. Jägerschmidt mit Anwendung von Äthylätherextraktion und Resorcin hat sich als die empfindlichste Caramelnachweismethode, die mit Aceton als weniger empfindliche herausgestellt. Die reagierende Substanz bildet wahrscheinlich in beiden Methoden das ω-Oxymethylfurfurol, ein Nebenprodukt der Caramelisation. Daher tritt die Reaktion bei dem flüchtigen Destillationsprodukt D der Saccharose am stärksten auf. Die Reaktion wird durch die Couleur, weiter durch die Präparate A, B und C der Reihe nach immer schwächer hervorgerufen.

4. Die reagierenden Substanzen der weniger empfindlichen Methode von C. Amthor sind die gefärbten Caramelstoffe, nicht aber die flüchtigen Zersetzungsprodukte. Die Empfindlichkeit dieser Methode ist bei dem Produkt B (Caramelen 0,1%) und bei der Couleur 0,1% am größten.

5. Die Methode von V. Grießmayer und L. Aubry wurde mit einem Colorimeter quantitativ gestaltet. Die Ergebnisse sind bei Caramelmalz und bei der Couleur befriedigend.

6. Die G. Lichthardt-Methode ist für die käufliche Zuckerfarbe viel empfindlicher als für unsere Caramelprodukte und für das flüchtige Caramelisationsprodukt D.

7. Die M. Fradiss-Methode liefert mit unseren Caramelprodukten sowie mit der Couleur ganz unbefriedigenden Ergebnisse.

8. Die C. Crampton und F. Simons-Methode der Entfärbung der Caramellösungen gibt mit den Produkten A, B und C und mit der Zuckercouleur völlige Entfärbung bei gewissen Dosierungen von Adsorptionserden wie Tonsil, Floridin und Fullererde.

9. Die Untersuchung der C. Crampton und F. Simons-Methode mit Ätherextraktion zeigt, daß keines von unseren Caramelisationsprodukten sich mit Äther extrahieren läßt. Die Naturfarbe eines gealterten Roggenbranntweins ist dabei nur zu 27% extrahierbar.

10. Die Methode von A. Straub ergab eine Bildung von farbigen Niederschlägen mit allen untersuchten Caramelpräparaten sowie auch mit einigen Weingattungen.

11. Bei Anwendung der P. Carles-Methode unterlagen die Caramelprodukte keiner Entfärbung mit Eiweiß. Nur der gealterte Roggenbranntwein und einige Weingattungen wurden ziemlich stark entfärbt.

12. Die von A. Thit angegebenen Reaktionen basieren auf einem der Jägerschmidt-Methode ähnlichen Verfahren. Doch werden durch die Jägerschmidt-Methode wesentlich kleinere Konzentrationen des ω-Oxymethylfurfurol in der Lösung nachgewiesen.

13. Die Megalhães-Reaktionen werden durch flüchtige Caramelisationsprodukte hervorgerufen; sie können also die Anwesenheit nur dieser destillierenden Produkte in der Lösung nachweisen.

Caramels and Caramelization. Part 2.
Formation of Oligosaccharides in the
Pyrolysis of Sucrose, by Shozaluo
Kitaoka and Kiroku Suzuki

INTRODUCTION

When sucrose is heated and melted, it turns into a brown substance, named "caramel", which has been chemically studied for some years, as described in the review of Truhaut et al., (1) in detail.

In 1858 Gelis (2) et al. reported that three anhydrosugars existed in sucrose caramel, as caramelan, caramelen and caramelin. His work formed the basis for further investigations of carmel. Stolle (3), Cunningham and Doree (4) studied caramelan in detail because it was supposed to be the primary product of the caramelization of sucrose. According to them, caramelan was an amorphous substance obtained by heating at about 190°, extracted with 84% ethanol, and formed by the loss of two moles of water per one mole of sucrose. Its properties, derivatives and reactivities were also reported in detail.

However, little chemical investigation of caramel was done after that, (5) and especially investigations in which modern analytical techniques were used were hardly reported. Hodge (6) indicated in his review of browning reaction that modern investigations were quite scarce, and the mechanism of caramelization of sugar has not been revealed at all. In the amino-carbonyl reaction which is considered to be the most representative browning reaction of sugar,

the elucidation of the reaction mechanism has made great progress, since 1-amino-1-deoxyfructose containing amino acid was found to be the initial intermediate. On the other hand, no clue has been found to elucidate the caramelization reaction. Therefore, we undertook the study of carmelization and found that caramelan, which had been believed to be the primary product of the caramelization of sucrose and a simple substance, was actually a mixture of a number of dehydration and condensation products of sucrose (7).

The present investigation partly revealed the composition of the extract, which was obtained when sucrose was heated and melted under the conditions of preparation of caramelan and the residue was extracted with 84% ethanol. This paper reports that after decomposition of sucrose to glucose and fructose or after transglycosilation a few glucodisaccharides were identified in the dehydration and condensation products.

Experimental Method

1) Preparation of Sucrose Caramel

Sucrose was heated and melted according to the method which was used to prepare carmelan by Cunningham and Doree(4). That is to say, dried sucrose in an open flask was heated at $190 \pm 5°$ in an oil bath. Sucrose was stirred with a glass rod and heated until it lost 12% of its weight. The syrup obtained was extracted with 5 parts of 84% hot ethanol. The extract was filtered after cooling and the filtrate was concentrated to a solid. A brown and hygroscopic powder was obtained. This was used as a sample of caramel. In order to prepare caramelan from it, it is dissolved in water and fermented by yeast to remove ferment sugar and then yeast is removed by filtration. The filtrate is condensed to dryness and caramelan is thus obtained.

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2) Fractionation of Sucrose Caramel

A caramel obtained from 100 g sucrose as mentioned above in 10% aqueous solution was absorbed onto a column (8) (10 X 55 cm) of activated charcoal-Celite (1:1, w/w) and the column was eluted with water, 2.5% and 5.0% ethanol solutions successively. Eluates, 2-3 liters in volume, were concentrated and examined by paper and thin-layer chromatographies for the contained substances and the eluates that gave qualitatively identical composition were combined to make a fraction. Ten fractions, I-X were obtained as shown in Fig. 1. When no spot appeared in the chromatogram, the concentrations of ethanol in eluent were changed as described above, and elution was continued. Elution is still being continued with 7.5 and 10.0% ethanol solutions and the results obtained from elution with up to 5.0% ethanol are reported in this paper.

3) Paper Chromatography

Ascending technique was employed, and if necessary, double or triple development was applied. The developing solvent systems used were as follows.

(a) n-propanol - ethyl acetate - water (7:1:2, v/v/v)

(b) n-butanol - pyridine - water (3:1:1, v/v/v)

(c) water-saturated phenol

The coloring agents were as follows:

(a) ammoniacal silver nitrate (a 1:1 mixture of 0.1 N silver nitrate and

5 N aqueous ammonia)

(b) benzidine reagent (a solution of 0.5 g benzidine and 10 g trichloroacetic acid in 100 ml of 90% n-butanol)

(c) periodate-permanganate reagent (9)

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4) Thin-layer Chromatography

A thorough mixture of 1 g of Silicalayer G (Nakarai Chemicals Co.) and 2 ml of 0.1 N boric acid was spread in 0.25 mm thickness. The plate was dried at 110° for 30 minutes and used.

The developing solvents were as follows:

(a) n-butanol – pyridine – water (5:3:2)

(b) n-butanol – acetic acid – water (5:1:4)

The staining reagents were as follows:

(a) ammoniacal silver nitrate

(b) naphtharesorcinol-sulfuric acid

(a 1:1 mixture of 0.2% naphtharesorcinol in ethanol and 20% sulfuric acid)

(c) anthrone-phosphoric acid (10)

5) Acetylation

Fractions of sucrose caramel were completely dried on phosphorus pentoxide, and acetylated by the reaction with acetic anhydride in cold pyridine (11). Acetates which were obtained as crystals were recrystallized and their physical constants measured. The melting point was measured by SHIMAZU micromelting point measurement apparatus and was not corrected.

Experimental Results

Paper and thin-layer chromatograms of caramelan did not give a simple spot, but revealed a great number of close spots in a belt that were almost indistinguishable from each other, as reported previously (7). So did sucrose caramel in this experiment. 5-Hydroxymethylfurfural was also detected as in the case of caramelan.

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Figure 1 shows a thin-layer chromatogram of fractions of caramel
aqueous solution obtained by eluting the charcoal-Celite column in the
way described above. The eluting solvents were water, 2.5 and 5.0% ethanol
solutions. Total yield of the fractions shown here was about 30% of the
caramel used, and the remaining parts are considered to be contained in
eluates with 7.5%, 10.0% and higher concentration ethanol and in the parts
which cannot be eluted.

Fifteen compounds in these fractions were found as shown in Fig. 1
and identification of them was attempted. Identification was carried out
mainly by paper and thin-layer chromatographies and partly by isolation of
the acetates.

Spot No. 1 was positive to silver nitrate reaction but negative to
ketose reaction (anthrone-phosphoric acid reagent) and gave the same Rf-
value as the glucose reference on the paper chlomatogram shown in Fig. 2;
thus it was identified as glucose. The physical constants and infrared
spectrum of the crystal isolated from the acetates of fraction I corresponded
to those of ⍺-D-glucose pentaacetate, which supports the identification.
The compound obtained by deacetylation of the acetates with sodium methoxide,
of course, indicated the same Rf-value as No.1 compound.

Spot No. 2 was positive to silver nitrate reaction and ketose reaction,
and gave the same Rf-value as ketose on the paper chromatogram (Fig.2); this
substance was identified as fructose.

Spot No. 3 was negative to silver nitrate reaction but positive to
periodate-permanganate reaction. As shown in Fig. 2, it gave the same Rf-value
as levoglucosan on the paper chromatogram and so it was identified as levo-
glucosan. The acetates obtained from this substance formed crystals, m.p.
105-108°, and no decrease of melting point was observed by mixed melting with
levoglucosan triacetate (m.p. 108°)[12]

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Their infrared spectra showed absolutely the same absorption.

Spot No. 4 was postive to naphthoresorcinol reaction, but negative to anthrone-phosphoric acid reagent and to ammonical silver nitrate reagent, and so it was considered not to be a reducing sugar. Because of the slight yield, no other identification experiment could be carried out. Although several spots of little yield were found besides No. 4 in fraction IV, none of them except No. 5 was reducing and could be identified.

Spot No. 5 was positive to silver nitrate but negative to ketose reaction. From the acetylation of fraction VI crystalline acetate was obtained, and paper and thin-layer chlomatographies of the deacetylized product showed that the crystal was derived from the substance of spot No. 5. The acetate has the physical constants shown in Table 2, and they correspond to those of β-isomaltose octa acetate (13). Mixed melting showed no decrease of their melting point and their infrared spectra were absolutely the same. From these facts the substance of spot No. 5 was concluded to be isomaltose.

Spot No. 6 was negative to the usual silver nitrate reagent but positive to stronger ammonical silver nitrate reagent (14) (prepared by adding the concentrated ammonia solution to 5% silver nitrate solution and dissolving the produced precipitate again), and positive to ketose reaction. Its Rf-value was the same as the sucrose reference on the chromatogram with all solvents (Fig. 3). From these facts the substance was concluded to be unchanged sucrose.

Spot No. 7 was negative to usual and high concentrated silver nitrate reagent but positive to anthrone-phosphoric acid reagent. Therefore, this substance is supposed to be the dehydrated product derived from fructose, but as the reference compound was not at hand it could not be identified further.

Spot No. 8 was positive only to naphthoresorcinol-sulfuric acid reagent and negative to any other coloring reagents. The structure of this compound is not known so far.

Spot No. 9 was positive to silver nitrate but negative to ketose reaction. The paper and thin-layer chromatographies of fraction VI showed the same Rf-value of Spot No. 9 as the reference kojibiose (Fig. 4) and so the substance of spot No. 9 was identified as kojibiose.

Spot No. 10 was positive to naphthoresorcinol-sulfuric acid reagent but negative to other reagents. This compound could not be identified.

Spot No. 11 was positive to ketose reaction but negative to even concentrated silver nitrate. Therefore, it was inferred to be a dehydrated product from fructose as spot No. 7.

Spot No. 12 was positive to silver nitrate but negative to ketose reaction. On the paper and thin-layer chromatograms of fraction VII and reference nigerose, they showed the same Rf-value with all solvents systems used. Therefore, this substance was identified as nigerose (Fig. 5).

Spot No. 13 and Spot No. 14: 1 g of syrup obtained by condensation of fraction IX was dissolved in water and it was fractionated again by activated charcoal-Celite column to separate the compound of spot No. 13 and that of Spot No. 14. A 5.0 X 50 cm column was used and gradient elution (15) with from 2.5% to 10.0% ethanol solution was used. Both compounds were completely separated in 3 1 eluents.

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Both fractions were condensed, dried and then acetylized, and the crystalline acetates were obtained. Physical constants of both acetates are shown in Table 3.

When both acetates were deacetylated and then hydrolized with 0.1 N sulfuric acid at 100° for 1 hour and the products were examined by thin-layer chromatography, the spots of glucose and another unknown spot were obtained in both cases. Neither fructose nor levoglucosan was detected in these hydrolized products. From these data the substances of No. 13 and No. 14 were inferred to be condensed substances of fructose and other dehydrated products of unknown structure.

Spot No. 15 was negative to the all reagents for reducing sugar but positive only to naphthoresorcinol-sulfuric acid reagent. The structure of this substance is also unknown.

Discussion

As seen in experimental results, sucrose caramel, prepared under the conditions of preparation of caramelan, was a mixture of a number of compounds. The presence of glucose and fructose means that a part of the sucrose was hydrolized during pyrolysis. Apparently the amount of fructose was less than that of glucose, because ketose would undergo secondary decomposition faster than glucose.

Among 15 substances that were relatively clearly distinguished, identified substances were levoglucosan and three kinds of glucodisacchrides, such as isomaltose, kojibiose and nigerose as well as glucose, fructose, and unchanged sucrose. Levoglucosan is 1, 6-anhydro-β-glucose, which is formed by the loss of one mole of water per one mole of glucose.. It is not

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clear whether sucrose splits into glycose and fructose at first during pyrolysis and then glucose turns to levoglucosan by dehydration, or if the glucoside moiety of sucrose changes directly to the hydration product on splitting of sucrose. It's very interesting that all of three identified glucosacchrides have α-glucosidic linkage. In the fractionation of this experiment (with up to 5.0% ethanol) most of the expected glucodisaccharides should be eluted, but no spot of glucodisaccharide could be obtained on the chromatogram besides the above-mentioned three glucosaccharides. In a caramel obtained by Sugisawa et al. by heating glucose at 150°, a number of glucodisaccharides with both α- and B-glycosidic linkages were identified. Therefore, the fact that glucodisaccharides of only α-glucosidic linkage were found might mot indicate that these saccharides were formed by the secondary dehydration condensation of the glucose formed by hydrolysis of sucrose, but it suggests a possibility that the α-glycosidic moiety of sucrose is trans-glycolated to freed glucose. However, as gentiobiose and sophorose were found in the hydrolysate of a pyrodextric made by roasting amylose (17), it cannot be concluded that this is the case, as there is no evidence for the selective formation of glucodisaccharides of α-glucosidic linkage from freed glucose.

Color reactions revealed a few ketose-containing substances. As reference compounds of ketose, including expected dianhydrodifructose, were not available, these ketoses could not be identified.

In addition several compounds, which seemed to be nonreducing, were detected on chromatograms. These were levoglucosan or other glucose anhydrides (e.g., dianhydroglucose (18), a nonreducing anhydride of fructose,

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and a disaccaride anhydride formed from nonreducing anhydride and sucrose glucose like the substances of spot No. 13 and spot No. 14. Such compounds are known to be formed by thermal polymerization of levoglucosan.(19)

In summary, the caramel obtained by heating sucrose at $190 \pm 5°$ and extracted with 84% ethanol was submitted to fractionation by chromatography. Unchanged sucrose, hydrolysis products of glucose and fructose, dehydration products of levoglucosan, and three ⱥ-glucodisaccharides of isomaltose, kojibiose and nigerose, were identified. The presence of two ketose-containing substances and a number of nonreducing substances, including anhydro-dihydro-saccharides made of glucose and anhydrosugar, was revealed as well.

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カ ラ メ ル の 研 究　第2報

ショ糖の熱分解による少糖類の生成

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Caramels and Caramelization. Part 2. Formation of
Oligosaccharides in the Pyrolysis of Sucrose

は じ め に

ショ糖を加熱熔融すると，いわゆるカラメルと呼ばれる褐色の物質になるが，このものの化学的本態については古くから研究があり，TRUHAUT ら[1] の総説に詳述してある。1858 年，GELIS [2] はショ糖カラメルの中に三種のショ糖脱水物が存在すると報告した。すなわちカラメラン，カラメレン，カラメリンである。この研究は以後のカラメル研究の足がかりとなり，STOLLE[3]，CUNNINGHAM and DORÉE[4] はさらに詳細な研究を行ない，とくにカラメランはショ糖のカラメル化における第一次生成物と考えられたので詳しく記載された。これらの結果を総括すると，カラメランは 190° 前後でショ糖を転熱し，84%エタノールで抽出してえられる無定形物質で，その組成はショ糖1分子より水2分子が脱水したものである。このものの性質，誘導体，反応性についても詳しく報告されている。

しかしカラメルの化学的本態に関する研究はその後少なく[5]，とくに近代的な分析技術を用いた報告は極めてまれであって，HODGE [6] は糖類の褐変反応の総説の中にカラメルの近代的研究が余りにも少ないことにおどろいている。そして糖類のカラメル化反応については，その機構が全くわかっていないのが現状である。糖類の関与する褐変反応の中で最も代表的なものと考えられる，いわゆるアミノーカルボニル反応ではアミノ酸を含む1ーアミノー1ーデオキシーフラクトースが初期中間生成物であることが見出されてから，その機構解明に長足の進歩が見られたが，カラメル化反応についてはそのような手がかりは何も見出されていない。この点にかんがみ，われわれはさきに従来ショ糖のカラメル化において第一次生成物であり，単一な物質と考えられていたカラ

メランが，多数の糖脱水物および結合物の混合物にすぎないことを示した[7]。

本研究はカラメランを調製する条件でショ糖を加熱熔融し，84%エタノールで抽出したものについて組成の一部を明らかにしたものであって，ショ糖がカラメル化に当ってグルコースとフラクトースに分解し，または transglycosylation によって，それぞれの脱水生成物，結合生成物を与えることを示し，若干のグルコ二糖類の同定に成功したので報告するものである。

実 験 方 法

1) ショ糖カラメルの調製

CUNNINGHAM, DORÉE [4] がカラメラン調製に用いた方法によってショ糖を加熱熔融した。すなわち，ショ糖を乾燥後開放したナス形フラスコに入れ，190 ± 5°の油浴中で加熱した。内容物はガラス棒でカク拌し，内容物の重量が12%減少する点で加熱を止めた。内容物は褐色のシラップとなり，これを5倍量の84%エタノールで加熱抽出した。冷後沪過し，沪液を濃縮して固形物に至らしめた。褐色の吸湿性のある粉末である。このものを試料カラメルとした。このものより所謂カラメランを調製するには，水に溶解した上酵母処理により酵母醱酵性糖を除き，酵母を沪別し濃縮して粉末化すればよい。

2) ショ糖カラメルの分画

100 g のショ糖よりえられた上記カラメルの10%水溶液を活性炭ーセライト（1：1，W/W）のカラム[4]（10×55cm）に吸着させ，水，2.5%エタノール，5.0%エタノールで順次溶出した。溶出液は2ー5ℓのフラクションとし，各フラクションは濃縮後ペーパークロマトグラフィ，薄層クロマトグラフィによって存在する物質を検出し，同様のスポットを与えるフラクションは合
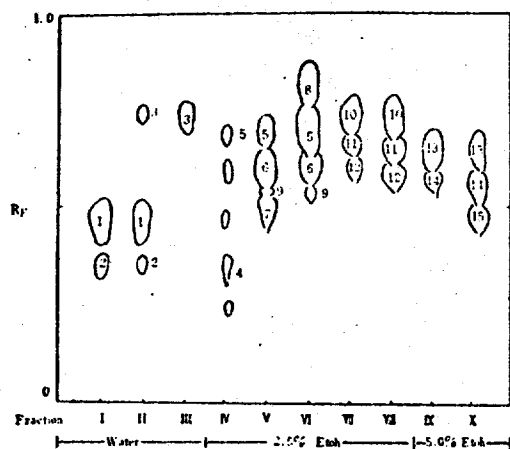
Fig. 1

Thin-layer chromatogram of Fractions（0—5％ ethanol eluates）of the 84 ％ ethanol extract of sucrose caramel

　　Solvent system : n-butanol-pyridine-water

　　（5:3:2）

　　Spray reagent : naphthoresorcinol-sulfuric acid

し，第1図に示すようにI—Xの10個の分画をえた。ク ロマトグラフィによって同じスポットがえられなくなる と溶出液のエタノール濃度を上記のように変えて溶出を つづけた。本試料はさらに7.5％ および 10.0％ エタノ ールにより溶出を続行しているが，本報告では5.0％エ タノール溶出までの結果について記載する。

3）ペーパー・クロマトグラフィ

一次元上昇法により，必要に応じて二重あるいは三重 展開を行なった。展開溶媒は次の通りである。

　(a)　n—プロパノール—酢酸エチル—水（7：1： 2，V，V，V）

　(b)　n—ブタノール—ピリジン—水（6：1：4， V，V，V）

　(c)　水または フェノール

発色剤は次の通りである。

　(a)　アンモニア性硝酸銀（0.1N AgNO₃ と濃Nアン モニア水を等量混合したもの）

　(b)　ベンジジン試薬（ベンジジン0.5 g，トリクロロ 酢酸 10 g を 80 ml の n—ブタノールと 15 ml の水 に溶かしたもの）

　(c)　過ヨウ素酸—過マンガン酸カリ試薬[9]

4）薄層クロマトグラフィ

薄層は Silicalayer G（半井化学製） 1 g に対し，2 ml の割合で 0.1N ホウ酸を加えよくカク拌後 0.25 mm の厚さにつくった。これを 110° に 30 分間乾燥したもの を使用した。展開剤は次のものを用いた。

　(a)　n—ブタノール—ピリジン—水（5：3：2）

　(b)　n—ブタノール—酢酸—水（5：1：4）

また発色剤はつぎの通りである。

　(a)　アンモニア性硝酸銀

　(b)　ナフトレゾルシノール—硫酸（0.2％のナフトレ ゾールシノール—エタノール溶液と20％硫酸の等 量混合物）

　(c)　アンスロン—リン酸試薬[10]

5）アセチル化

ショ糖カラメルを分画後五酸化リン上でよく乾かし， 冷ピリジン中で無水酢酸と反応させアセチル化を行なっ た[11]。アセチル化物で結晶にえられたものは再結晶の 上，物理恒数を測定した。融点は島津微量融点測定器に よって測定し，補正はしていない。

実 験 結 果

カラメランは前に報告した[7]ように，濾紙 および薄 層クロマトグラフィで単一のスポットを与えず，帯状に なって相互に分離確認できないほど多数のスポットが密 接して検出される。本実験で用いたショ糖カラメルにつ いても全く同様であった。5—ヒドロキシメチルフルフ ラールのスポットはカラメランの場合と同様に確認され た。

試料カラメルの水溶液を前述の方法によって活性炭— セライトを用いるカラム・クロマトグラフィによって分 画し，フラクションを整理結合したものの薄層クロマト グラムが第1図である。溶出を水および 2.5％ と 5.0％ エタノールで行なった結果だけをここに示している。こ こに示した分画の収量合計は試料カラメルの約30％にあ たり，残部は7.5％ および 10.0％エタノールおよびさ らに高濃度アルコール溶出分画中と不溶性部にある。

第1図に示したように，これら分画中に18の物質の存 在が確認される。そのおのおのの物質の同定を試みた。 同定は主として濾紙および薄層クロマトグラフィによ り，アセチル化物による同定もあわせ行なった。

スポット No. 1：このものは前述還元性でケトーズ 反応（アンスロン—リン酸試薬）陰性であり，No.2に 示すように濾紙クロマトグラム上既知のグルコースと同 一の Rf を与え，グルコースと同定された。また分画I をアセチル化したものから結晶がえられ，このものの分 解点あるいは融点は第1表に示すように α—D—グルコース・・・・ペンタ
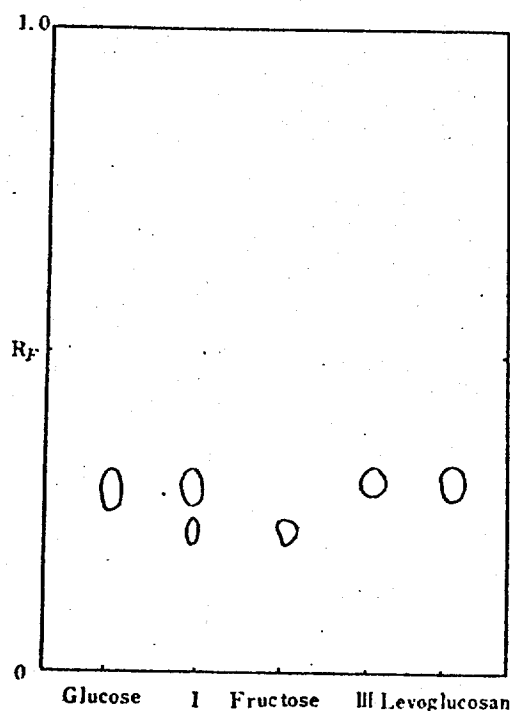
Fig. 2

Paper chromatogram of Fractions I and III
Solvent system :
n-propanol-ethyl acetate-water（7:1:2）
Spray reagent :
ammoniacal silver nitrate; NaIO₄-KMnO₄

Table　1

	Spot No. 1	α-Glucose Pentaacetate
M.p., °C	108–112	112–113
$[\alpha]_D$ (CHCl₃)°	+100	+103

アセテートの物理恒数[11] と一致し，赤外吸収曲線も一致したのでこの同定を支持するデータとなった。このアセチル化物をメタノール性ナトリウムで脱アセチル化したものは勿論 No. 1 と同じ R_F のスポットを与える。

スポット No. 2：このものは硝酸銀還元性でケトース反応陽性であり，沪紙クロマトグラフィでフラクトースと同一の R_F を与えた（第2図）。このことから，この物質はフラクトースと同定された。

スポット No. 3：このものは硝酸銀反応陽性で，過ヨウ素酸一過マンガン酸カリで陰性であった。第2図に示すように沪紙クロマトグラフィでレボグルコーザンと

同一の R_F を与え，レボグルコーザンと同定された。このものをアセチル化すると融点 105—108° の結晶を与え，このものはレボグルコーザン・トリアセテート（融点 108–109°)[12] と混接して融点の降下を示さなかった。また赤外スペクトルでも両者は全く同一の吸収を示した。

スポット No. 4：ナフトレゾルシノール—硫酸反応は陽性であるが，アンスロン—リン酸試薬およびアンモニア性硝酸銀試薬には陰性で，還元性ではないと考えられるが，収量が少なく，これ以上の同定実験が行なえなかった。分画 IV はこの他多くの収量の少ないスポットがえられたが，スポット No. 5 以外はすべて非還元性で同定されなかった。

スポット No. 5：硝酸銀を還元し，ケトース反応陰性である。分画 VI はアセチル化したものから結晶性アセチル化物がえられ，その脱アセチル化物の沪紙および薄層クロマトグラムから，このものがスポット No. 5 の物質より誘導されたことがわかった。このアセチル化合物は第2表に示す物理恒数を示し，標準品の β-イソマルトース・オクタアセテートの物理恒数[13] と一致した。両者は混融して融点の降下を示さず，また赤外スペクトルでも完全に一致した。このことからスポット No. 5 はイソマルトースであると結論される。

Table　2

	Spot No. 5	β-Isomaltose Octaacetate
M.p., °C	142–145	146–147
$[\alpha]_D$ (CHCl₃)°	+98	+96.9

スポット No. 6：このものは通常の硝酸銀試薬非還元性であるが，さらに強いアンモニア性硝酸銀[14]（5% AgNO₃ に濃アンモニア水を滴下し，一旦生じた沈でんを溶解させたもの）溶液には陽性の反応を示し，またケトース反応も陽性であった。薄層クロマトグラム上シュクロース標準品とすべての溶媒系で同一の R_F を示した（第3図）。このことから，このものは未分解のシュクロース（ショ糖）と結論される。

スポット No. 7：硝酸銀試薬に対しては通常および高濃度の条件で陰性であり，アンスロン—リン酸試薬には陽性であった。このことから，この物質はフラクトースの脱水生成物であると想像されるが，標準品をもたなかったので，これ以上の同定はできなかった。

スポット No. 8：ナフトレゾルシノール—硫酸試薬にのみ陽性で，他の呈色試薬にはすべて陰性であった。このものの構造は現在の段階ではわかっていない。

V Sucrose	Ⅵ Kojibiose	Ⅶ Nigerose
Fig. 3	Fig. 4	Fig. 5
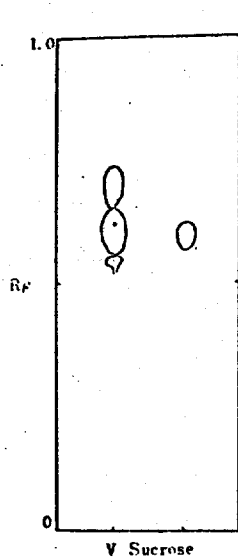
Fig. 3
Thin-layer chromatogram of
Fraction V
Solvent system :
　n-butanol-pyridine-water
　(5:3:2)
Spray reagent :
　5 % AgNO₃-conc. NH₄OH
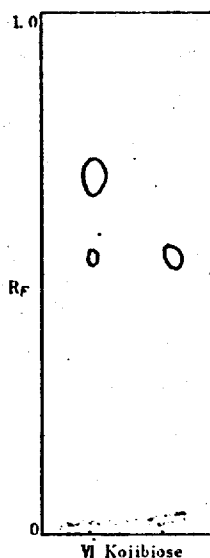
Fig. 4
Thin-layer chromatogram of
Fraction Ⅵ
Solvent system :
　n-butanol-pyridine-water
　(5:3:2)
Spray reagent :
　ammoniacal silver nitrate
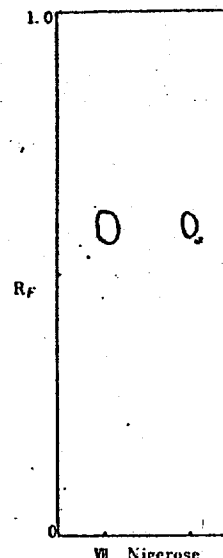
Fig. 5
Paper chromatogram of
Fraction Ⅶ
Solvent system :
　n-propanol-ethyl acetate
　-water (7:1:2)
Spray reagent :
　ammoniacal silver nitrate

スポット No. 9 ： 過酸銀還元性でケトース反応陰性であった。このものを含む分画Ⅵを沪紙および薄層クロマトグラフィで標準品のコージビオースと比較したところ，同一の Rf を与えた（第4図）。このことから，スポット No. 9 はコージビオースと同定された。

スポット No. 10 ： ナフトレゾルシノール―塩酸試薬には陽性で，他の試薬には陰性であった。このものの同定には成功していない。

スポット No. 11 ： ケトース反応陽性で塩銀溶液は濃度の条件でも還元しない。この結果からスポット No. 7 と同様にフラクトースの脱水生成物と推定される。

スポット No. 12 ： 過酸銀還元性で，ケトース反応陰性である。分画Ⅶを標準品のニゲロースと沪紙および薄層クロマトグラフ上比較して，用いたすべての溶出系で同一の Rf 値を示した。この物質はよってニゲロースと同定された。（第5図）

スポット No.13 および No.14 ： 分画Ⅸを濃縮してえたシラップ1 g をとり，水溶液とし，活性炭―セライトカラムによる再クロマトグラフィを行ない，両者の分別を試みた。カラムは5.0×12 cm で溶出には2.5 %から 10.0 % までのエタノールを用いる gradient elution によった。全溶出液3 ℓで両者を完全に分離することができ

た。

両フラクションをそれぞれ濃縮乾固したのちアセチル化して，それぞれ結晶性のアセチル化物をえた。両アセチル化物の物理恒数を第3表に示す。

Table 3

	Spot No.13	Spot No.14
M.p., °C	118-119	165-166.5
[α]D (CHCl₃)	+19	+52

両アセチル化物を脱アセチル化し，0.1 N の塩酸で 100° 1時間加水分解したものについて薄層クロマトグラフィーでしらべたところ，両物質ともグルコースのスポットのほかに未知のスポットがそれぞれえられた。フラクトース，レボグルコーザンはこの加水分解物に存在しない。このようなデータから，スポット No. 13 および14の物質はグルコースと他の糖類または脱水生成物が結合したものであると推定される。

スポット No. 15 ： 過酸銀に対する反応はすべて陰性で，ナフトレゾルシノール―塩酸試薬にのみ陽性であ

った。このものの構造もわかっていない。

考　察

　実験結果からわかるように，カラメランをつくる条件で調製したショ糖カラメルは多数の物質を含む複雑な混合物である。これらのうちグルコースとフラクトースが存在することはショ糖の一部が熱分解中に加水分解することを示している。この両者の見掛けの量をくらべてフラクトースが少ないのはケトースの方がより速やかに二次分解を行なうことを示すものと考えられる。

　比較的明確に分離出来た15個の物質のうちで，グルコースおよびフラクトースおよび未反応のショ糖以外に同定しえたのはレボグルコーザンとイソマルトース，コージビオースおよびニゲロースの3種のグルコ二糖類であった。レボグルコーザンは1，6—アンヒドロ—β—グルコースであって，グルコースより水1分子の脱水によって生成する。ショ糖が熱分解時に先ずグルコースとフルクトースに加水分解し，ついで前者が脱水してレボグルコーザンを生成するのか，あるいはショ糖の開裂によって直ちにグルコース部分がこの無水物に変化するのかはわからない。次に同定しえた3種のグルコ二糖類はすべてα—結合を有するものであることは極めて興味深い。この実験で取扱ったカラメルの分別（5.0％エタノール画分まで）では予想しうるグルコ二糖類の大部分が溶出されるはずであるが，クロマトグラム上の呈色反応の結果から上記3種以外にグルコ二糖類と推定されるスポットはえられなかった。杉沢ら[16]はグルコースを150°に加熱してえたカラメルから多種類のグルコ二糖類を同定しており，これらはα—結合のものもβ—結合のものも含んでいる。この点から，本実験でα—結合を有するグルコ二糖類のみが見出されたことは，ショ糖が加水分解して生成したグルコースから二次的脱水縮合によってこれら二糖類ができたのではなく，ショ糖中のα—グルコース部分が transglycosylation によって遊離グルコースと結合した可能性が考えられる。しかしアミロースを乾熱してえられるピロデキストリン中にはゲンチオビオース，ソホロースの存在が知られている[17]ので，この推定が正しいと断定することはできない。ただ一旦遊離したグルコースからα—結合の二糖類だけが選択的に生成すると考えるのは困難である。

　ケトースの呈色をする物質は比較的少数しかえられていない。予想されるジアンヒドロ—ジフラクトースを含めてケトース関係の標品化合物がなかったので，これら物質の同定は現段階では行なえなかった。

　以上のほかに非還元性と見なされる若干の物質がクロマトグラム上に検出されている。これらはレボグルコー

ザンまたはその他のグルコースの無水物（たとえばジアンヒドログルコース[18]），フラクトースの非還元性無水物のほか，スポット No.13，14で明らかになったような非還元性無水物と糖グルコースよりなる二糖類無水物がかなり生成していることを示している。このようなタイプの化合物はレボグルコーザンの熱重合反応によって生成することが知られている[19]。

　以上要約すると，ショ糖を 190±5° に加熱してえたカラメルを84％エタノールで抽出したものから，クロマトグラフィによる分別によって未分解のショ糖，加水分解産物のグルコース，フラクトース，脱水生成物のレボグルコーザンのほか，イソマルトース，コージビオース，ニゲロースの3種の α—グルコ二糖類を同定した。この他ケトース誘導体と考えられる物質2種と，非還元性物質若干の存在を明らかにした。この後者には糖無水物とグルコースよりなる二糖類無水物が含まれる。

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Summary

Sucrose was pyrolyzed in the open air at $190 \pm 5°$ to 12 % weight loss and the residue extracted with 84 % hot ethanol. The caramel obtained by concentrating this extract was submitted to carbon-Celite column chromatography and the fractions of $0-5.0$ % ethanol eluates analyzed for the constituents by means of paper and thin-layer chromatographies. Glucose and fructose were found to be present, the fact indicating that a part of sucrose was hydrolyzed during the pyrolysis. Levoglucosan and the three α-gluco-disaccharides of isomaltose, kojibiose and nigerose were identified by chromatographies and conversion into the acetates. Color reactions revealed presence of a few ketose-containing substances, beside undecomposed sucrose, and a number of unreducing substances, some of which were proved to be anhydro-disaccharides made of glucose and an anhydrosugar.

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Quantitative Determination of Caramel

In Wine, Distilled Spirits, Vinegar, and Vanilla Extract

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A combined qualitative and quantitative method is presented by which caramel can be precipitated and separated from other coloring matter in wine, distilled spirits, vinegar, and vanilla extract. The identity of the caramel can be corroborated by known methods and the intensity of color determined by a tintometer. By use of tintometer readings, the caramel can be calculated as per cent of color or per cent by weight or volume in a liquid.

CARAMEL is extensively used as a coloring agent in beverages and food products. Manufactured by heating sugar or hydrolyzed starch until the sweet taste is destroyed and a uniform, dark-brown, viscous mass is formed, caramel is said to be a carbohydrate, having the formula $C_{12}H_{18}O_9$, (6). Von Elbe (8) after investigating sucrose caramel stated that "caramel consists of a mixture of colorless compounds and a dark-brown, humic" substance which shows the properties of a lyophobic acid". That statement has been corroborated in this laboratory and work on caramel derived from other sugars shows that most varieties which have been thoroughly dried are insoluble in water, acid solutions, and alkaline solutions.

The principal varieties in commerce are acid proof, nonacid proof, foaming, malt, and baker's and confectioner's caramels. Another variety is sometimes found in concentrated grape must, or grape concentrate which has been heated at such a high temperature during evaporation of the juice that some of the sugar has caramelized. Large quantities of grape concentrate are used in the manufacture of wine, and since Internal Revenue laws prohibit the presence of any form of caramel in standard wine, it is necessary to have a method for detecting all varieties of caramel.

The literature contains many methods for detecting caramel in beverages and food, but no quantitative method. The experience in this laboratory, where a large part of the work involves wine, is that none of the published tests is satisfactory for all kinds of wine and distilled spirits, particularly grape concentrate and the wine made from it. The fact that caramel may be made partly insoluble in acid and alkaline solutions suggested the possibility of using such a reaction as the basis for a method for obtaining caramel free from other coloring matter. Common reagents such as ethers, alcohols, etc., which are used in some of the known methods, were tried without success; the caramel was not completely precipitated or it contained other coloring matter. Some of the chemicals, such as tartrates and sulfites, which are present in wine or are used in its manufacture were tried and it was found that the combinations used in the present method precipitated all the caramel without including other coloring matter.

The method was developed primarily for the analysis of wine, but is applicable to distilled spirits, vinegar, and vanilla extract. Since the caramel is removed completely and in a pure state from the liquid by the method of analysis, and from a caramel-free liquid no color is removed, a brown precipitate is proof of the presence of caramel. If desired, its identity can be corroborated by known tests. For a quantitative determination a colorimetric method is considered most suitable and a Lovibond tintometer is used for the purpose.

All tintometer readings were made at a north window, using daylight reflected from an opal glass plate. All readings of more than 20 brown were made on diluted solutions, except the standard caramel solutions which were read in a 0.156-cm. (1/16-inch) cell and calculated to a 1.25-cm. (0.5-inch) cell. Although manufacturers use a 2.5-cm. (1-inch) cell for readings of color in the analysis of caramel, analytical laboratories use a 1.25-cm. (0.5-inch) cell as the standard for color readings of alcoholic liquors and other liquids colored by caramel. The Lovibond tintometer has been criticized on the ground that readings cannot be made in one cell and calculated to a cell of another thickness and that the slides are inaccurate. The figures in Table III show that the Lovibond slides are accurate for readings up to 20 brown and the figures in the example below show that calculations can be made on readings in cells of different thicknesses. Beyer (4) has shown that caramel solutions follow Beer's law and that readings with the Lovibond tintometer are as accurate as those made with a photometer.

Allen (2) states in connection with color work on indigo, "The most satisfactory solution has been found by employing the Lovibond tintometer as the color-measuring instrument. Since the relative proportions of red, yellow, and blue will vary in different shades, the measure of depth must be taken as the total number of color units obtained by adding together the units of red, yellow, and blue, given by the glasses required to match the pattern. There is a definite relation between the percentage weight of indigo on the material and the tintometric reading." The principle of adding color units and making calculations with them applies to caramel as well as to indigo. Unless it is colorless, all wine free from caramel contains brown color as well as red. This is shown in Table IV by the readings of the original wine, every one of which contains brown. Even varieties which appear to the eye to be pure red require brown slides to match the color, a striking example being sample 140,500, a dark red claret which contains almost as much brown as red. If caramel is added to wine, the caramel recovered by the method of analy-

sis, and its color determined, the sum of the brown and red slides of the Lovibond scale divided by the sum of the brown and red slides for the reading of the original sample containing caramel multiplied by 100 equals the per cent of caramel color in the total color, because the readings are in units of color and they can be compared as any other units. To illustrate the method for calculating the per cent of caramel in a liquid, the following example is given, using a dark red Burgundy wine to which caramel was added and recovered by the method of analysis, the color readings being made in a Lovibond tintometer.

A. Wine
4.0 brown
5.4 red in $^1/_{16}$-inch cell $\approx$ 32.0 brown
43.2 red in 0.5-inch cell

B. Wine and water
22 ml. of wine and 3 ml. of water.
3.5 brown
4.4 red in $^1/_{16}$-inch cell $\approx$ 28.0 brown
35.2 red in 0.5-inch cell

C. Caramel and water
3 ml. of standard caramel solution and 22 ml. of water.
12.0 brown
0.8 red in 0.5-inch cell

D. Wine and Caramel
22 ml. of wine and 3 ml. of standard caramel solution
5.0 brown
4.6 red in $^1/_{16}$-inch cell $\approx$ 40.0 brown
36.8 red in 0.5-inch cell

E. 3 ml. of caramel added to 22 ml. of wine and recovered by the method of analysis. Final solution diluted to 25 ml. for reading.
10.5 brown
0.6 red in 0.5-inch cell

Reading of B + reading of C = reading of D, wine and caramel before analysis.
28.0 brown
35.2 red + 12.0 brown
0.8 red = 40.0 brown
36.0 red

Reading of B + reading of E = reading of wine and recovered caramel
28.0 brown
35.2 red + 10.5 brown
0.6 red = 38.5 brown
35.8 red

F. There is a reduction of 12.5% of color in the method; therefore the reading is corrected by dividing by 87.5 and multiplying by 100.
10.5 brown
0.6 red ÷ 87.5 × 100 = 12.0 brown
0.7 red

Reading of B + reading of F = reading of wine + reading of caramel recovered by analysis.
28.0 brown
35.3 red + 12.0 brown
0.7 = 40.0 brown
35.9 red = D after analysis

The reading 12.0 brown
0.7 red or total of 12.7 units ÷ 40.0 brown
36.8 red or total of 76.8 units × 100 = 16.5%. The caramel color is 16.5% of the total color in the wine containing caramel.

As an example of the method for calculating the approximate per cent by weight or volume of caramel in a liquid, suppose that a sample from a 50-gallon barrel of brandy is analyzed by the method and the color reading of the recovered caramel is 10.6 brown
0.4 red a total of 11.0 units in a 0.5-inch cell of the Lovibond tintometer.

Correcting for the 12.5% reduction in color due to the method

$$11.0 \div 87.5 \times 100 = 12.6 \text{ units}$$

Assume that the caramel in the brandy had the composition of the average shown in Table I. Since 0.10 gram per 100 ml. of the average caramel reads 9.6 brown
0.6 red a total of 10.2 units, the quantity required to read 12.6 units is

$$10.2 : 12.6 = 0.10 : X \qquad X = 0.123 \text{ gram}$$

The total solids of the average caramel, 67.5%, is equivalent to a specific gravity of 1.33. The volume of caramel per 100 ml. which has a reading of 12.6 total units is

0.123 ÷ 1.33 = 0.092 ml.
0.092 ml. per 100 ml. is per cent by volume
0.092% of 1 gallon or 128 fluid ounces = 0.118 fluid ounces
0.118 × 50 = 5.9 fluid ounces of caramel in the 50-gallon barrel of brandy

Table I. Analysis of Caramel Samples as Received from Manufacturers

Sample No.	Mfgr.	Variety	Moisture Solids, % by Weight		Color Reading of 0.10 Gram of Caramel in 100 Ml. of Water 0.5-Inch Cell
140,409	A	No claim	25.9	74.1	11.0 brown 0.8 red
141,730	A	No claim	37.7	62.3	11.0 brown 0.8 red
140,414	B	Acid-resistant	31.1	68.9	9.5 brown 0.8 red
141,729	B	No claim	38.1	61.9	9.0 brown 0.8 red
140,458	C	Malt	29.3	70.7	9.0 brown 0.8 red
140,461	D	Acidproof	22.1	77.9	11.0 brown 0.8 red
141,770	D	Foaming	31.3	68.7	11.0 brown 0.4 red
141,747	E	Bakers and confectioners	29.4	70.6	9.0 brown 0.8 red
140,471	E	No claim	34.2	65.8	8.5 brown 0.8 red
141,766	F	Extra strong	34.1	65.9	11.0 brown 0.4 red
141,767	F	Foaming	41.8	58.2	11.5 brown 0.4 red
141,832	G	Not acidproof	37.2	62.8	8.0 brown 0.4 red
140,413	G	Acid-resistant	27.7	72.3	8.0 brown 0.4 red
140,396	H	No claim	28.4	71.6	8.5 brown 0.4 red
140,397	I	No claim	28.1	71.9	7.5 brown 0.4 red
140,463	J	No claim	43.7	56.3	9.0 brown 0.8 red
		Av.	32.5	67.5	9.6 brown 0.6 red

Internal Revenue regulations permit a maximum of 6 fluid ounces of caramel to be added to 50 gallons of brandy. Therefore, in the brandy of unknown origin mentioned above the quantity of caramel is within the limits of the regulations.

In addition to the chemical reagents used in the method, there is another substance necessary for the precipitation of caramel the nature of which is at present unknown. It is found in wine, vinegar, and vanilla extract so far analyzed, but is not present in distilled spirits or caramel solutions and it must be furnished from some other source, for without it, caramel cannot be recovered quantitatively by this method from distilled spirits and caramel solutions. If a caramel in a 10 to 21% alcohol solution only is analyzed by the method for wine, not more than 5% of the caramel is recovered because of lack of the ingredient mentioned.

In the search for a substance which might contain this necessary precipitating material, several varieties of sugar were tried. When dextrose was used, the recovery of caramel was still only 50%, with honey 65%, with sucrose 75%, with sorbitol or mannite 85%, with maple sugar 90%, and with light brown granulated cane sugar 100%. All grades of brown sugar do not contain caramel. The one used in this work was obtained in one-pound packages at a grocery store; it was found to be caramel free and very light in color, comparable to Mulliken's Color Chart A, yellow tint 2 (7). It should be tested by the method of analysis to determine its freedom from caramel, by the use of 3 grams in enough 10 to 21% alcohol to make a volume of 25. During the search for this ingredient, gelatin and its hydrolytic products were studied, but it was found that they precipitate not only all of the caramel but also wine and fruit colors. Although sometimes used as a clarification medium in wine manufacture, gelatin has never been noticed in wine because of its insolubility in alcoholic liquids. Pectin, when added to wine formed during the analysis a mass of stringy material or a curd

itate depending on the quantity added, which carried wine with it and ruined the determination. However, pectin ever been found in grape wine and only in small quantities few samples of abnormal fruit wine and in a concentrate from black grapes. If present in wine, it can be removed the caramel precipitate by the acetone-hydrochloric acid as described below. Dextrin in any appreciable quantity ound to collect on the sides of the precipitating cylinder as a sticky mass, but it was encountered only while endeavoring apt the method to the analysis of beer. The method can be for the determination of caramel in beer but it is most unactory and is not recommended.

STANDARD CARAMEL SOLUTIONS

r use in developing the method, 16 samples of caramel obd from 10 manufacturers were analyzed for moisture, solids, tinctorial strength in order to compare their quality. The orial strength was determined in a solution of 0.10 gram of nel in 100 ml. of water, using a 0.5-inch cell in a Lovibond meter, matching the color with brown slides series 52 and lides series 200. The results are shown in Table I.

lor is the only basis on which the various brands of comial caramel can be compared because they vary in moisture, s, ash, etc.; so if all the samples are adjusted to the same h of color they are comparable in quality. Since it was found diluted caramel solutions were decomposed by yeasts and ls, even when kept in a refrigerator, freshly prepared standsolutions were used in all determinations.

ich sample of caramel was diluted with sufficient water to l a solution of tinctorial strength corresponding to a reading $\frac{2.0 \text{ brown}}{0.4 \text{ red}}$ to $\frac{12.5 \text{ brown}}{0.8 \text{ red}}$ in a $^1/_{16}$-inch cell. This was adopted he standard and it made all the samples comparable. The dard solutions were analyzed for solids and ash (Table II). se figures may be of value in detecting adulterated caramel. sample is diluted sufficiently to have a color reading of 12.0 2.5 brown in a $^1/_{16}$-inch cell and its solid content is materially than the minimum shown in Table II, the inference is very ng that the sample contains color other than caramel.

Table II. Analysis of "Standard Solutions of Caramel"

(Color readings of 12.0 to 12.5 brown in $^1/_{16}$-inch cell)

mple No.	Mfgr.	Solids G./100 ml.	Ash G./100 ml.
140,409	A	0.6725	0.0186
141,730	A	0.6200	0.0130
140,414	B	0.6585	0.0122
141,729	B	0.6530	0.0231
140,458	C	0.8135	0.0056
140,461	D	0.7155	0.0190
141,770	D	0.6172	0.0250
141,747	E	0.7550	0.0200
140,471	E	0.7332	0.0025
141,766	F	0.6500	0.0672
141,767	F	0.5455	0.0358
141,832	G	0.7110	0.0070
140,413	G	0.9130	0.0100
140,396	H	0.9042	0.0062
140,397	I	0.9130	0.0625
140,163	J	0.6230	0.0320
	Av.	0.7169	0.0293

arious volumes of the standard solutions having color read-s of $\frac{12.0 \text{ brown}}{0.4 \text{ red}}$ to $\frac{12.5 \text{ brown}}{0.8 \text{ red}}$ in a $^1/_{16}$-inch cell were diluted 25 ml. and tintometer readings made in a 0.5-inch cell; 1.5 of such a solution diluted to 25 ml. read $\frac{6.0 \text{ brown}}{0.4 \text{ red}}$ in a 0.5-h cell. However, when 1.5 ml. of a standard solution were uted to 25 ml. and treated as in the last part of the method of lysis, beginning with the sentence "Transfer the paper and cipitate to a 150-ml. beaker." and finally read in a 0.5-h cell, the color was invariably $\frac{5.0 \text{ brown}}{0.2 \text{ red}}$ to $\frac{5.5 \text{ brown}}{0.4 \text{ red}}$ instead $\frac{6.0 \text{ brown}}{0.4 \text{ red}}$

This procedure shows that there was a reduction in color, due t to a loss of caramel precipitation in the main part of the thod but to the treatment of the precipitated caramel after was removed from the rest of the sample. The reduction in

Table III. Color Reading of Standard Caramel Solutions Diluted with Water to 25 Ml.[a]

Volume of Solution, Ml.	Color Reading	
	Untreated solution, 0.5-inch cell	Treated solution, 0.5-inch cell
0.75	$\frac{3.0 \text{ brown}}{0.2 \text{ red}}$	$\frac{2.5}{0.2}$ to $\frac{2.75 \text{ brown}}{0.2 \text{ red}}$
1.5	$\frac{6.0 \text{ brown}}{0.4 \text{ red}}$	$\frac{5.0}{0.2}$ to $\frac{5.5 \text{ brown}}{0.4 \text{ red}}$
3.0	$\frac{12.0 \text{ brown}}{0.8 \text{ red}}$	$\frac{10.0}{0.6}$ to $\frac{11.0 \text{ brown}}{0.8 \text{ red}}$
6.0	$\frac{23.0}{1.0}$ to $\frac{24.0 \text{ brown}}{1.4 \text{ red}}$	$\frac{20.0}{1.0}$ to $\frac{22.0 \text{ brown}}{1.4 \text{ red}}$

[a] Standard caramel solutions were treated by the last part of the method of analysis beginning with "Transfer the paper and precipitate to a 150-ml. beaker". An average of 12.5% reduction in the color reading was caused by the treatment.

color, averaging 12.5%, is characteristic of the method, and is consistent as shown by the readings in Table III and the other tables.

DETERMINATION OF CARAMEL

METHOD FOR WINE. *Reagents.* Powdered boric acid, U.S.P. Powdered citric acid, U.S.P.

Powdered potassium bitartrate, c.p. Powdered tartaric acid, c.p.

Powdered sodium bisulfite, reagent grade, minimum 95% NaHSO₃ (not sodium metabisulfite)

Sodium hydroxide, c.p. Ether, c.p. Acetone, c.p. Alcohol, U.S.P.

Boric-Citric Acid Solution. To 100 ml. of 95% alcohol, add 6 grams of boric acid and 2 grams of citric acid, warm to 55° C. for solution, and cool to room temperature.

Precipitating Solution. To 56 ml. of the alcoholic boric-citric acid solution in a 100-ml. cylinder, add 19 ml. of ether and 25 ml. of acetone and mix. Prepare just before use.

Alcoholic Sodium Hydroxide Solution. To 100 ml. of alcohol which is practically aldehyde-free add 4 grams of sodium hydroxide dissolved in 4 ml. of water, mix, filter, and keep in a tightly stoppered bottle.

Apparatus. Glass-stoppered cylinder graduated to 100 ml. and capable of holding 130 ml.

Procedure. Place 25 ml. of wine, containing 10 to 21% of alcohol by volume and not more than 20% of solids, in the specified glass-stoppered cylinder. Add in the order named, shaking after each addition, 0.3 gram of potassium bitartrate, 0.1 gram of tartaric acid, and 0.4 gram of sodium bisulfite. Allow the mixture to stand for 10 minutes and add 100 ml. of the precipitating solution. Shake the cylinder vigorously for 1 or 2 minutes, removing the stopper every 15 or 20 seconds to release the pressure and stand overnight for complete precipitation of the caramel. Place in a Gooch crucible a mat of paper pulp $^1/_{16}$ inch or less in thickness, rinse it with alcohol using suction, then decant through it the liquid in the cylinder until approximately 10 ml. remain. Mix the liquid and precipitate in the cylinder and pour rapidly through the Gooch. Rinse the cylinder several times with 5 to 10 ml. of alcoholic boric-citric acid solution, pouring the rinsings through the Gooch. Wash the precipitate in the Gooch with 25 ml. of hot alcoholic boric-citric acid solution (heated to boiling in a 600-ml. beaker on an electric hot plate to prevent ignition and swirling the beaker constantly to prevent bumping), followed by 10 ml. of 95% alcohol, then 10 ml. of 4% alcoholic sodium hydroxide solution, and finally by 10 ml. of 95% alcohol.

Transfer the paper and precipitate to a 150-ml. beaker, and wash the Gooch with 5 ml. of 0.5 N aqueous sodium hydroxide solution, followed by 15 ml. of water, adding the washings to the beaker. Test with litmus paper to be sure the solution is alkaline. Boil vigorously for several minutes to dissolve the caramel, stirring constantly to prevent bumping, and cool gradually to room temperature. Filter the contents of the beaker through a 9-cm. filter paper which has been wet with water, collecting the filtrate in a 50-ml. cylinder. Wash with water until the filtrate is colorless and measures approximately 23 ml., make faintly acid with N hydrochloric acid, and complete the volume to 25 ml. with water. Read the color of the caramel solution in a 0.5-inch cell in the Lovibond tintometer, correct it for the 12.5% manipulation loss, divide it by the color reading of the original wine, and multiply by 100 for the per cent of caramel color in the total color.

The analysis of grape concentrate for caramel coloring is more difficult than that of wine itself because the concentrate contains all the solids and vegetable color originally present in the grape

juice, much of which is removed in the process of manufacturing wine. The concentrate must be diluted with 10 to 21% alcohol and filtered before analysis. Difficulty may be experienced in filtering the concentrate even after diluting 2 to 5 ml. of the sample to 25 ml. For this reason 2 ml. of the concentrate are generally preferred. The use of suction or a centrifuge may not aid the filtration. The best procedure is to dilute 4 ml. of the concentrate to 50 ml. with 10 to 21% alcohol, add 1 gram of purified talc U.S.P., mix, and filter through a dry double filter paper. It may take several hours for this filtration, but the liquid must be clear; otherwise the method is a failure. Purified talc U.S.P. is inert and no caramel will be adsorbed by it when the quantity used is small.

Some varieties of very dark colored grapes produce concentrate which is very different in composition from the ordinary varieties. It is almost black in color and, because of being unfermented, contains an excess of iron which, during the analysis, precipitates an iron color complex noticeable as a red contamination in the caramel. The coloring matter is not removed by the regular method of analysis and a special procedure is necessary to eliminate it. No wine or ordinary grape concentrate has been analyzed which caused trouble and this extra step does not need to be taken unless the caramel precipitate is abnormal because of its color or other unusual appearance which will be apparent to an experienced analyst. The procedure is as follows:

Prepare an acetone–hydrochloric acid mixture containing 25 to 29% acid by volume by pouring 15 ml. of c.p. acetone in a 25-ml. cylinder and adding hydrochloric acid (1.18 sp. gr.) to the 20- or 21-ml. mark. Pour the mixture into a small beaker, cool it in ice water to 20° to 25° C., and use it immediately at that temperature. This volume, which is sufficient for one test, should be measured accurately because experiments have proved that caramel is soluble in stronger acid. However, the mixture of the strength specified will break up the iron color complex without dissolving the caramel as precipitated by the method. At the point in the method of analysis, beginning with the sentence "Transfer the paper and precipitate to a 150-ml. beaker.", fill the Gooch half full of the acetone–hydrochloric acid mixture, continue the suction until a drop of liquid is drawn through, discontinue the suction for a minute to allow the liquid to penetrate the precipitate, suction off the liquid, and wash thoroughly with alcohol. Repeat the process using alcoholic sodium hydroxide and wash with alcohol.

Replace the Gooch in the suction flask, with a second one containing a mat of paper pulp and with a small stirring rod transfer the mat and precipitate from the first Gooch to the second one. Fill the second Gooch half full of the acetone–hydrochloric acid mixture and, while holding the mat against the side of the cru-

cible with a stirring rod, break it up with a second rod, without disturbing the lower mat. When it is disintegrated and saturated with the acetone–hydrochloric acid mixture apply suction, draw the liquid through, add the remainder of the mixture, suck it dry, wash with alcohol, discontinue the suction, add alcoholic sodium hydroxide, stir the loose pulp, suck it dry, and wash with alcohol. Place the mat and precipitate in a 150-ml. beaker, and wash both Gooch crucibles with 5 ml. of 0.5 N aqueous sodium hydroxide solution, followed by 15 ml. of water, adding the washings to the beaker. From this point follow the regular method. If it is necessary to reduce the volume of the caramel solution evaporate it in an alkaline condition to less than 25 ml., make slightly acid, and complete the volume to 25 ml.

Table IV. Analysis of Grape, Berry, and Fruit Wine before and after Addition of Caramel
(0.5-inch cell)

Sample No.	Wine	Alcohol % by volume	Color Reading of Original Wine	Color Reading of Final Solution after Analysis of Original Wine	Volume of Standard Solution of Caramel Added to Original Wine Ml.	Color Reading Final Solution Representing Recovered Caramel
139,476	Muscatel	20.0	7.5 brown 1.8 red	Colorless	1.5	5.5 brown 0.4 red
139,425	Muscatel	20.6	15.0 brown 10.0 red	Colorless	3.0	10.5 brown 0.8 red
137,827	Muscatel	18.2	5.75 brown 0.6 red	Colorless	0.75	2.6 brown 0.2 red
141,587	Muscatel	15.5	11.5 brown 0.6 red	Colorless	6.0	21.0 brown 1.2 red
141,696	Muscatel	19.5	22.5 brown 1.6 red	Trace	1.5	5.75 brown 0.6 red
134,428	Burgundy	12.0	24.0 brown 65.0 red	Colorless	1.5	5.5 brown 0.2 red
137,280	Burgundy	13.0	32.0 brown 96.0 red	Colorless	0.75	2.75 brown 0.2 red
140,878	Zinfandel	13.0	16.0 brown 103.0 red	Colorless	1.5	5.5 brown 0.2 red
140,309	Claret	13.5	16.0 brown 17.2 red	Colorless	3.0	10.0 brown 0.6 red
142,212	Chianti	13.1	12.0 brown 29.2 red	Colorless	0.75	2.75 brown 0.2 red
139,253	Port	23.6	16.0 brown 25.6 red	Colorless	3.0	10.0 brown 0.6 red
140,388	Tawny port	20.0	18.0 brown 4.0 red	Colorless	3.0	10.0 brown 0.6 red
139,182	Port	20.5	25.6 brown 29.6 red	Colorless	1.5	5.25 brown 0.4 red
140,337	Sherry	20.4	14.0 brown 1.4 red	Trace	1.5	5.25 brown 0.2 red
140,339	Sherry	20.0	18.0 brown 2.0 red	Colorless	3.0	10.0 brown 0.6 red
140,384	Sherry	20.5	10.0 brown 0.4 red	Colorless	0.75	2.6 brown 0.2 red
140,382	Sherry	20.25	16.0 brown 1.5 red	Trace	1.5	5.5 brown 0.5 red
142,736	Sherry	20.2	12.5 brown 0.8 red	Colorless	1.5	5.25 brown 0.4 red
139,475	Malvasia	21.4	16.0 brown 11.6 red	Colorless	6.0	20.5 brown 1.2 red
140,842	Angelica	20.8	15.0 brown 1.8 red	Colorless	3.0	10.5 brown 0.6 red
142,450	Tokay	20.2	7.5 brown 3.8 red	Colorless	0.75	2.75 brown 0.2 red
139,100	Alicante	20.6	18.0 brown 20.0 red	Colorless	1.5	5.5 brown 0.4 red
140,387	Chablis	12.8	5.5 brown 0.4 red	Colorless	0.75	2.6 brown 0.2 red
140,839	Sauterne	13.4	10.0 brown 0.8 red	Colorless	1.5	5.25 brown 0.4 red
141,920	Apple	20.0	7.0 brown 0.4 red	Colorless	3.0	10.25 brown 0.8 red
141,922	Loganberry	13.0	11.0 brown 43.2 red	Colorless	1.5	5.0 brown 0.4 red
141,921	Blackberry	13.0	26.0 brown 20.8 red	Colorless	0.75	2.6 brown 0.2 red
141,926	Currant	13.0	12.0 brown 9.6 red	Colorless	1.5	5.0 brown 0.6 red
148,169	Cherry	21.0	11.0 brown 2.2 red	Colorless	3.0	10.25 brown 0.8 red
140,555	Orange	19.0	3.0 brown 0.2 red	Colorless	1.5	5.0 brown 0.4 red
142,135	Peach	20.1	4.5 brown 0.2 red	Colorless	3.0	10.5 brown 0.6 red

THOD FOR DISTILLED SPIRITS. *Reagents.*
acetate, C.P. Ammonium chloride, C.P.
ercial brown sugar (sucrose), granulated,
sl-free.

gents and apparatus listed under wine
is.

edure. Place 25 ml. of the spirits in a
tory funnel, add 50 ml. of ether, shake
asly for 1 minute to remove the alcohol,
o settle for at least 15 minutes, draw off
er aqueous layer into another separatory
, and discard the ether layer. Add to
queous solution 25 to 35 ml. of ethyl
:, shake vigorously for 1 minute to re-
ncharred oak tannin, allow to settle for
t 15 minutes, draw off the lower layer
e specified glass-stoppered cylinder, add
as of brown sugar, fill to the 21-ml. mark
ater, and complete the volume to 25 ml.
5% alcohol. Mix the contents of the
er to dissolve the sugar and proceed as
d for wine. After the final solution has
cidified and made up to a volume of 25
d 0.07 gram of ammonium chloride, mix,
ow to stand for at least 30 minutes. If
innin from redwood or uncharred oak
s in the solution, it will be precipitated
ammonium chloride. Filter through a
ilter paper, read the color of the filtrate
5-inch cell, and calculate the per cent of
l in the original spirits as described
wine.

THOD FOR VINEGAR. *Procedure.* Place
of vinegar in the specified glass-stoppered
er and add 3 ml. of 95% alcohol. Pro-
s directed for wine. Make the final
a up to 22 ml. for the color reading and
te the per cent of caramel in the original
r as described under wine.

THOD FOR VANILLA EXTRACT. *Procedure.*
nl. of vanilla extract, add 25 ml. of water
gram of purified talc and mix. Prepare
of paper pulp in a Gooch crucible using
1, and filter the vanilla mixture through
ral times until a layer of talc is formed
e filtrate is clear. Place 25 ml. of the
in the specified glass-stoppered cylinder
oceed as directed for wine. Make the final volume up to
and multiply the color reading by 2 or make it up to 12.5
l use the direct reading. Calculate the per cent of caramel
original vanilla extract as described under wine.

Table V. Analysis of Grape and Raisin Concentrates and Wine

(0.5-inch cell)

Sample No.	Grape and Raisin Concentrates	Concentrate before dilution	Final solution after analysis of diluted concentrate	Caramel calculated to undiluted concentrate
			Color Reading	
141,691	Raisin concentrate (5 ml. diluted to 25 ml. with 20% alcohol for analysis)	200.0 brown 12.0 red	3.5 brown 0.4 red	17.5 brown 2.0 red
137,872	Grape concentrate (same dilution)	375.0 brown 20.0 red	5.0 brown 0.4 red	25.0 brown 2.0 red
140,826	Red grape concentrate (same dilution)	20.0 brown 50.0 red	Colorless	....
140,827	Red grape concentrate (same dilution)	150.0 brown 200.0 red	Colorless	....
140,828	White grape concentrate (same dilution)	100.0 brown 6.0 red	Colorless	....
140,829	Red grape concentrate (same dilution)	40.0 brown 50.0 red	Colorless	....
	Overcooked Grape Concentrate[a]			
136,947	5 ml. concentrate diluted to 25 ml. with 20% alcohol	575.0 brown 40.0 red	30.0 brown 2.4 red	150.0 brown 12.0 red
136,947	2 ml. concentrate, diluted to 25 ml. with 20% alcohol	575.0 brown 40.0 red	11.5 brown 1.0 red	140.0 brown 12.5 red

Wine Made from Concentrate 136,947

Wine		Alcohol by Volume, %	Color Reading before Analysis	Color Reading after Analysis
138,192[b]	Red port, no dilution	20.9	42.0 brown 9.6 red	5.0 brown 0.6 red
137,009[c]	Red port, no dilution	20.8	40.0 brown 8.8 red	3.5 brown 0.6 red

[a] Sample 136,947 was overcooked and contained a considerable quantity of caramel. The manufacturer blended it with wine in such small proportions he thought the color added by the caramel would be of negligible value and could not be detected. Two lots of blended wine, samples 138,192 and 137,009, were analyzed, the caramel was detected, and the quantity determined. The caramel color reading of the wine corrected for the reduction of 12.5% in the method, divided by the caramel color reading of the concentrate, corrected, multiplied by 100 equals the per cent of concentrate used in the wine.
[b] For sample 138,192, the calculation is
(5.0 + 0.6) ÷ 87.5 × 100 = 6.4 corrected color reading for wine.
(150.0 + 12.0) ÷ 87.5 × 100 = 185.1 corrected color reading for concentrate.
6.4 ÷ 185.1 × 100 = 3.5% of concentrate in wine.
[c] For sample 137,009, a similar calculation shows presence of 2.5% of concentrate in wine.

DISCUSSION OF THE METHOD

sample should be clear, filtered if necessary. If a sample
than 25 ml. is used, it should be diluted with alcohol and
so that the volume for analysis is 25 ml. containing 10 to
lcohol. The percentage of water is as important as that
other reagent. Most inorganic salts, other than the re-
listed, such as common laboratory chemicals, are detri-
as they tend to disturb the chemical balance, preventing
te separation of the caramel and precipitating an excess
reagents. Normal wine, distilled spirits, vinegar, and
extract do not contain such salts in sufficient quantity to
re with the method.

ratio between the reagents used in the method is very
a and the quantities of all of them should be carefully
ed, as a material change would interfere with the proper
tation of the caramel. The quantities used are satisfac-
r all types of dry and sweet wine, distilled spirits, vinegar,
or vanilla extract. The difference in acidity between eastern
California wine was taken into consideration in fixing the
ity of tartaric acid to be used and the normal sulfite content
: in fixing the quantity of sodium bisulfite. The potassium
tate, tartaric acid, and sodium bisulfite with the boric and
icids not only aid in the precipitation of the caramel, but
m a mat on which the caramel settles, permitting its easy
d. The alcoholic boric-citric acid solution acts as a sol-
r sugar and other solid matter; the more sugar there is in

the sample the less cream of tartar and tartaric acid are precipi-
tated, a point being reached at which neither reagent is precipi-
tated and a sirupy mass is thrown down which makes the removal
of the caramel from the cylinder exceedingly difficult. There-
fore, the solids in the sample being analyzed should not exceed
20%.

All of the samples of caramel reduced Fehling's solution be-
cause of the presence of sugar, but in this method, all sugar is re-
moved by the use of the alcoholic boric-citric acid solution and in
no instance has the final caramel solution after analysis reduced
Fehling's solution. Because of this fact, in the final step of the
analysis the precipitated caramel can be boiled in aqueous solu-
tion with alkali to dissolve it without any possibility of increas-
ing the caramel.

All caramel color readings should be made in a solution which
is slightly acid because the color is darker in an alkaline than in
an acid condition, this darkening being due to a large increase in
red color with only a slight increase in the brown. If it is de-
sired to evaporate a caramel solution after precipitation by this
method, it should be done in an alkaline condition because non-
acid proof caramel may be precipitated in a liquid which is more
than slightly acid.

APPLICATIONS

Many varieties of wine, including grape, berry, and fruit, were
analyzed by the method and when no caramel was present, the
final solution was colorless, except in a few instances when there
was a trace of color, which it was impossible to remove by any
variation in the method. In other samples of the same varieties
of wine, the final solutions were colorless. For these reasons it
was concluded that the slight color was due to a small quantity

Table VI. Analysis of Distilled Spirits before and after Addition of Caramel

(0.5-inch cell)

Sample No.	Variety	Proof	Marsh Test (²)	Cyclohexanol Test (5)ᵇ	Color Reading of Original Spirits	Color Reading of Final Solution after Analysis of Spirits	Standard Solution of Caramel Added to Original Spirits, Ml.	Color Reading of Final Solution Representing Caramel Recovered
131,905	Whisky	100.0	Negative	Negative	15.0 brown 0.4 red	Colorless	1.5	5.0 brown 0.4 red
131,353	Brandy	100.0	Negative	Negative	10.0 brown 0.2 red	Colorless	3.0	9.75 brown 0.8 red
139,934	Scotch	86.8	Positive	Positive	8.5 brown 0.4 red	3.0 brown 0.2 red	3.0	13.0 brown 0.8 red
137,974	Rum, domestic	86.0	Negative	Negative	7.0 brown 0.4 red	Colorless	0.75	2.6 brown 0.2 red
101,989	Rumᵃ, imported	87.0	Positive	Positive	45.0 brown 4.0 red	16.0 brown 1.0 red	....	....
142,029	White oak chips	100.0	Positive	Negative	6.0 brown 0.2 red	Colorless	0.75	2.5 brown 0.2 red
142,028	Redwood chips	100.0	Trace	Trace	9.0 brown 1.4 red	Colorless	6.0	20.0 brown 0.8 red

ᵃ Labeled "Imported West Indies Rum, carefully distilled and aged 2.5 years in charred white oak casks". Rum was very dark in color and contained coal-tar dye, caramel, and oak wood color. Coal-tar dye extracted from rum in wool dyeing test was reddish-brown. Final solution containing caramel after analysis of rum was tested for coal-tar dye and result was negative, showing that dye in rum did not interfere with method for caramel.

ᵇ The cyclohexanol test is similar to Marsh test, but has the advantage that color from uncharred oak goes into the upper layer, whereas with the Marsh test the color from uncharred oak goes into the lower layer the same as caramel. The reagent is composed of 50 ml. of cyclohexanol, 50 ml. of methyl propyl ketone, 3 ml. of sirupy phosphoric acid .85°, and 3 ml. of distilled water.

recovery of the caramel was obtained. The Scotch whis contained caramel and wl 1.5 ml. of a standard solution caramel were added to it, final solution after analy contained all the added cara as well as that originally in whisky.

Since most wine is stored redwood containers and tilled spirits in oak, it was sired to know what effect extractive matter of those c tainers would have on analysis of liquor stored them. Oak and redwood cl were soaked in 50% alcohol til the liquids were dark cause of the color extract The two liquids were th analyzed by the method, each case the final solution colorless, showing that neit oak color nor redwood color terferes with the separation

of caramel incorporated in the wine by the use of caramelized concentrate as a blending agent. This conclusion was verified by combining and concentrating several of the solutions containing the color and applying the phenylhydrazine test (3) to them. In every instance a precipitate was obtained. Table IV shows the results of the analysis of the caramel-free wine, the volume of standard caramel solution added to the wine, and the tintometer reading of the recovered caramel. The smallest addition of standard caramel solution was 0.75 ml. and the largest 6.0 ml. in enough wine to make a volume of 25 ml. for analysis. The maximum of 6 ml. was taken because that dilution makes a liquid as dark as would ordinarily be used in any commercial wine. When a definite volume of a standard solution of caramel was added to each sample of wine, the color reading of the final solution containing the recovered caramel was the same as that of the standard caramel solution made up to 25 ml. after treatment as shown in the last column of Table III. When corrected for the average reduction in color of 12.5%, the recovery was 100% except in the case of the foaming type which was 80%. Foaming caramel is different from other varieties in composition and properties and the present work indicates that it may contain less actual caramel color and more brown color which is not caramel, thus accounting for the lower recovery value.

Some of the samples of sherry, such as No. 140,339, were extremely dark for sherry, but analysis disclosed no caramel, proving that deep color does not necessarily mean caramelization as a result of the baking of the sherry.

Several samples of grape concentrate and one of raisin concentrate were analyzed by the method. Two of them were found to contain caramel and the others had none (Table V).

Several samples of distilled spirits were analyzed by the method and in every instance in which no caramel was present, the final solution was colorless. When a definite volume of a standard solution of caramel was added to each sample, full

caramel. When caramel was added, full recovery was obtai in each liquid.

Two samples of rum were analyzed, one of which was foun contain coal-tar dye, in addition to caramel. To investigate effect of dyes on the method, experiments were performed, u six kinds of coal-tar dyes and two of vegetable colors, all of wl made brown colored liquids in dilute solutions. Each of the ei colors was added to a sample of wine or spirits which was t analyzed. In every instance, the final solution was colorl showing that the particular coal-tar and vegetable colors u did not interfere with the method for caramel. The results of analysis of the distilled spirits are shown in Table VI.

Three samples of apple cider vinegar were analyzed by method and in each one, the final solution contained a trac color. Since the sweet cider and apple wine analyzed contai no caramel, it was concluded that the color in the vinegar was to a small quantity of caramel which was formed in the cool of the apple products before they were used in the vinegar p ess. This conclusion was verified by combining and concent

Table VII. Analysis of Vinegar and Vanilla Extract before and after Addition of Cara

(0.5-inch cell)

Sample No.	Material	Color Reading of Original Material	Color Reading of Final Solution after Analysis of Original Material	Standard Solution of Caramel Added to Original Material, Ml.	Color Reading Final Solutic Representin Recovered Car
140,362	Cider vinegar	10.0 brown 0.6 red	Trace	1.5	5.75 brown 0.4 red
141,945	Cider vinegar	13.5 brown 0.8 red	Trace	3.0	10.5 brown 0.6 red
142,278	Cider vinegar	14.0 brown 0.8 red	Trace	0.75	3.0 brown 0.2 red
142,191	Pure apple juice (plus 1 ml. of glacial acetic acid)	4.0 brown 0.6 red	Colorless	1.5	5.25 brown 0.4 red
131,658	Bourbon vanilla extract	136.0 brown 8.0 red	Colorless	1.5	5.25 brown 0.4 red
141,949	Tahiti vanilla extract	32.0 brown 1.6 red	Colorless	0.75	2.6 brown 0.2 red
141,948	Mexican vanilla extract	40.0 brown 1.6 red	Colorless	3.0	10.0 brown 0.6 red
141,947	Bourbon vanilla extract	48.0 brown 3.2 red	Colorless	6.0	21.0 brown 1.2 red

several of the solutions containing the color and applying phenylhydrazine test (5) to them. In every instance a precipitate was obtained.

Four samples of genuine vanilla extract were analyzed by the method and in every instance the final solution was colorless, showing the absence of caramel. The results are shown in Table I.

SUMMARY

Caramel in wine, distilled spirits, vinegar, and vanilla extract is precipitated by organic acids and solvents in a form easily freed from other coloring matter. The purified precipitate is caramel and its identity can be corroborated by the usual tests. The quantity is determined in a Lovibond tintometer by color readings which are very accurate up to 20 units and when the manipulation loss in the method is corrected, the results of analyses are also very accurate.

The use of analytical results in making calculations is shown in the examples given for (1) per cent of caramel color in the total color, (2) per cent by weight or volume of caramel in a liquid,

and (3) per cent of caramelized concentrate in a volume of wine (Table IV).

The method can be applied to all varieties of grape, fruit, and berry wine, distilled spirits, vinegar, vanilla extract, grape concentrate, and commercial caramel. It is not affected by substances usually present in those liquids, such as fruit and vegetable colors, wood extractive matter, or by certain coal-tar dyes.

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Annual poundage reported per substance by NAS and FEMA user firms

Appendix A Part I Substances 0001-0251 Preliminary data

Table 1

Caramel (NAS#0059 FEMA#2235)

Reports to NAS 1960/1970	Poundage reported to NAS		NAS ratio 1970/1960	Reports to FEMA	Poundage reported to FEMA 1970	Total 1970 lbs NAS + FEMA
	1960	1970				
79/93	19039550	22039485	1.2	75	942476	22981961

CARAMEL COLOR

★ Its Properties ★ And Its Uses

With these newly revealed points on the much-used but little understood coloring — its chemical nature, types, manufacture, and testing — you will be aided in proper selection and food application of the handy agent

FRED W. PECK

Vice-President, Sethness Products Co., Chicago
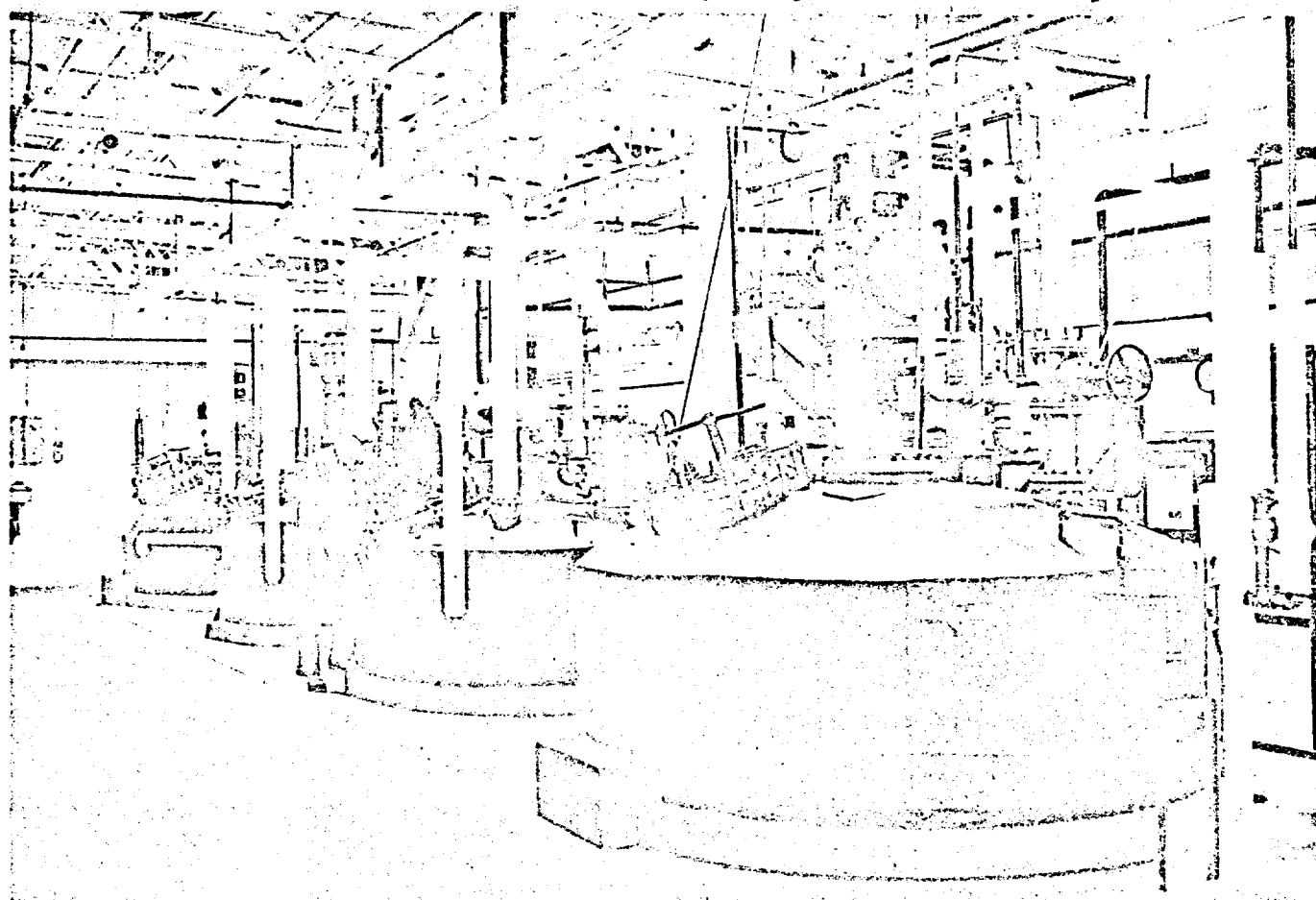
Banks of Cookers are on the top floor of the new plant. Here liquid corn syrup and other raw materials are processed to customer's specified shade of color. These stainless steel pressure kettles cook 5000 gallons at a time. Other vessels process 1,000 gallon batches.

CARAMEL COLOR
★ Its Properties   ★ And Its Uses

FRED W. PECK
Vice-President, Sethness Products Co., Chicago

Exactly how caramel is formed in the process of burning sugar has puzzled scientists for years. In fact, the reaction is still not completely understood.

However the baker, the bottler, the confectioner—and many other food processors—can benefit from the scientist's knowledge of caramel's pertinent properties and behavior.

Of immediate interest to the user are the characteristics and applications of the various available grades. Here, these will be pointed up. And mechanisms of the formations will be noted to indicate the many facets of the reactions.

In the case of caramel color, there seems to be a relationship between viscosity and the rate of solubility. Usually, less viscous caramels dissolve faster and have greater color stability, shelf life, and retention of complete solubility. Also, low viscosity caramel is handled with a minimum of waste and effort.

Every manufacturer seeks to reduce viscosity. Thus, some caramels have the same solids content and specific gravity as others, but flow more freely and have greater stability. As for standardization on this point, it is considered that a quality acid proof caramel color should weigh about 11 lb. per gal., and have a Baumé of 34.5-36.5 at 60°F. An ex-

ception to this is Double Strength Acid Proof Caramel, which normally has a Baumé of 27.0 to 30.0 at 60°F.

Another important property is pH. This varies with the type of caramel but gives an indication of its quality. High pH can indicate an incomplete burn or excessive alkali. Either or both of these conditions mean that the caramel's tinctorial (color) strength will increase as the color ages.

Above a pH of 6.0, the product is susceptible to mold. And at a pH of less than 2.5, it tends to resinify or gel within a short period. This process is accelerated as the pH drops below 2.5. For maximum stability, the pH of acid proof caramel color, undiluted, should be 2.8-3.3, with an

is not an easy matter for the average user to accurately determine color strength.

He has three alternatives: (1) buy the equipment to make the tests; (2) send the sample out to a chemical laboratory; or (3) take the word of a reliable manufacturer.

A spectrophotometer is an excellent instrument for measuring tinctorial power of caramel color, if the caramel user happens to have this instrument in the laboratory. The more sophisticated spectrophotometers are rather expensive to use as a control instrument unless the laboratory happens to have one for other uses. However, there are many good colorimeters on the market, which are good quality control instruments for determining tinctorial power of caramel. Any reliable colorimeter will permit a caramel user to check caramel shipments for uniformity of tinctorial strength.

Some colorimeters are based upon the same principle as the spectrophotometer and employ a diffraction grating which permits the operator to adjust the light source wave length to that of his choosing. Some, such as the Klett-Summerson Photoelectric Colorimeter, employ colored glass filters to regulate the wave length of the light source. Visual type of colorimeters use glass colored standards to which a solution of caramel is matched.

Any of these types of colorimeters can be successfully employed by the caramel user in a quality control program.

An interesting sidelight on caramel color is that color-spectrum analysis shows its composition to be about 70% red, 25% yellow, and 5% blue. But if certified colors are used in an attempt to duplicate the color of caramel, it requires 60% yellow, 35% red, and 5% blue. This apparent discrepancy seems to indicate that "certified red" is more intense than "caramel red." Therefore more yellow is needed to tone down the intensity of the red to make a synthetic color matching true caramel.

On a spectrophotometer or colorimeter, the best wave length for caramel color measurement is from 590 to 640 millimicrons. At this wave length the best correlation and reproducibility is found, even though by calculating percentages and wave lengths the measurement should be at approximately 650 millimicrons. This merely indicates once more

* Equation relating light transmittance (at given wavelengths) to concentrations and depths of solutions.

that caramel color is a chemical oddity. It is not a true solution and does not follow Beer's Law.* Thus, the variation between actual and theoretical wave lengths.

3 Other Tests

Further tests are carried out as follows:

1. Isoelectric Point—Dissolve 2.5g. of plain (unflavored) gelatin in 500 cc. of distilled water. Pour 100 cc. into each of four separate beakers and adjust pH of each beaker to 1.0, 1.5, 2.0, and 2.5, respectively with hydrochloric acid.

Then dissolve 0.5g. of the caramel to be tested in 500 cc. of distilled water. Pour 100 cc. into each of four beakers and adjust pH of each to 1.0, 1.5, 2.0, and 2.5, as above.

Fill four test tubes about ⅔ full from each of the marked beakers containing caramel solution. Add several drops of gelatin solution of the same pH as the caramel test solution, and observe immediately against a strong light. Look for a haze at the interface of the gelatin and caramel solutions. Repeat this procedure with other caramel solutions, always matching pH values of the test solutions. Let test tubes stand for 12 hr. and repeat observations.

This test is based on the known isoelectric point of gelatin, which is 4.7. At lower pHs, gelatin is a positive colloid and will be precipitated by the negative caramel, or remain clear if the caramel is also positively charged. By bracketing the isoelectric range, it is possible to establish that a caramel is not above a certain value. A haze at this pH with gelatin insures that it is below this figure.

Gelatin solutions should be made fresh each 24 hr., since they decompose on standing.

2. Colloid Character—Prepare 1.0% U.S.P. tannic acid solution and filter until clear. Make up 1.0% caramel solution in distilled water. To 13 cc. of caramel solution add 12 cc. of tannic acid solution. Mix well. Mixture should remain clear for 24 hours.

3. Acid Resistance—Make 1.0% caramel solution with distilled water. Dilute 50 cc. of this solution to 250 cc. and add 7.0 cc. of concentrated hydrochloric acid. Boil for 30 minutes, using a reflux condenser to eliminate loss of water during the boiling. Cool to room temperature and observe for cloudiness in ordinary light. Repeat after 24 and 48 hours. Solution should remain brilliant and clear through 48 hours.

How Made

Now that properties and uses have been described, how are the various caramels made?

Today's plant consists entirely of stainless steel equipment — kettles, lines, storage tanks, filters, etc. Recording thermometers automatically print a temperature record of each batch as it is processed. In fact, little is left to the human element, and complete laboratory control assures consistent uniformity.

High quality caramel color is manufactured from liquid corn sugar containing about 75 to 85 percent reducing sugars calculated as dextrose. Sugar is charged into scrupulously clean autoclaves or kettles. Two types of kettles are employed, depending on type of caramel to be processed.

One type, known as an "open kettle," ranges in size from 8,000-10,000 lb. capacity. The other is a "pressure kettle" which can hold 50,000 lb. of sugar. All kettles are equipped with large stainless steel rotary or turbine-type agitators, since it is necessary to "stir" the sugar constantly during the process.

Sugar is then heated in the presence of reactants and catalysts. During this process the temperature is held at over 250°F. for several hours. When the proper color is reached, contents of the kettle are quickly cooled to about 200°F., and put through a filter that has a capacity of 10,000 gal. per 8-hr. day.

After filtration, the caramel is quickly chilled to a temperature below 100°F. by passing it through a tube and shell heat exchanger en route to the final storage tanks. The quick chilling produces a more stable product.

Carmelizing of sugar is of course the very heart of the process. It is during this process that we encounter the term "straining the caramel". This implies too long a period of heating or too high a temperature used in an effort to produce a higher coloring strength. In either case the caramel is said to be "strained".

When this occurs, certain of the caramel's desirable properties are lost. Depending on extent of the straining it may lose a small or large degree of its usual stability. Overburning also results in a caramel with excessive viscosity. Apparently, there is a definite maximum of coloring strength which can be obtained in a given caramel, according

cient margin of safety.

Spirit caramel, used in whiskey, brandy, and cordials, should instantly dissolve in a 60% solution of alcohol and water by volume and give a sparkling brilliant solution. On the spectrophotometer, spirit caramel must have the proper balance of coloring tones to duplicate the color of aged whiskey.

This type should have an isoelectric point below a pH of 1.5, preferably 1.0, to keep it from precipitating any negative colloidal material such as tannin and vegetable extract. It must also have an extremely low iron content (a maximum of 10 to 15 ppm. is desirable) and be free of all traces of heavy metals.

There is still some market for beer color although not nearly as much as there was 20 years ago. The American public now favors a pale, dry beer. In England, however, breweries are probably the largest consumers of caramel. But here the methods of brewing differ entirely from those followed in this country and on the Continent.

Our brewmasters are principally of German descent and use that system. The British are addicted to employment of various sugars in the manufacture of their beer. In their processing they add large quantities of caramel color.

Beer caramel is somewhat weaker in tinctorial strength than most other caramels. It has a higher pH than acid proof caramel, 4.4 to 4.5. Caramels which give higher or lower pH when added to the fermenting wort can alter the pH sufficiently to change the flavor of the resulting beer.

They must be compatible with hops and all other materials used in brewing, carry a positive charge, and be able to withstand the fermentation process. Most important, they must be absolutely chill-proof and not cause a haze in the beer.

Baker's Types

In the modern bakery there is an ever present need for caramel color. The product principally maintains a uniform shade of coloring in baked goods. For flavoring reasons, a baker might want to use light colored cocoa or light rye flour. But his trade may be partial to dark colored goods. In this case, the caramel darkens his goods and pleases the consumer's eye as well as his palate.

Caramel is also extensively used by cookie and cracker bakers. In fact, it is employed in practically every instance when the manufacturer wishes to darken his baked products. There are many grades of baker's caramel coloring. Some are manufactured to sell at a very low price, while others are made for the more discriminating buyers. Obviously, cheap grades are not too carefully processed or refined.

Some are not clear and brilliant in solution, since they are not filtered or centrifuged as are the better grades. Many of the cheaper grades are not standardized for coloring power. Here, the baker must experiment with each new lot.

Although all baker's caramels appear to be heavy black syrups, nothing about their appearance is indicative of strength or quality. However, characteristics of a good baker's caramel are known. It weighs approximately 11 lb. per gal., is free flowing, and is low in viscosity.

This free-flow quality is very important, especially with baker's caramel. It facilitates solution in water or dispersion in dough, which is essential for uniformity of shade in the finished goods.

A water solution of a quality baker's caramel should be clear and have the natural caramel shade. It should have a pH of approximately 4.0 to 4.2 (near the normal fermentation pH) and a high tinctorial strength.

Caramel plays a role in the manufacture of soups, gravies, gravy flavor sauces, and condiments. In each case it must be tailored to suit these products. This also applies to caramel for prepared cake mixes, canned meat products, flavoring extract, household extracts, coffee products, bouillon cubes, dog food, and many other food items.

Testing for Color

Although cost of caramel in a finished product is small, amount of coloring per gallon may vary. Thus, tinctorial strength is of prime importance to the user. A few cents more per gallon may buy considerably more coloring strength. However, it
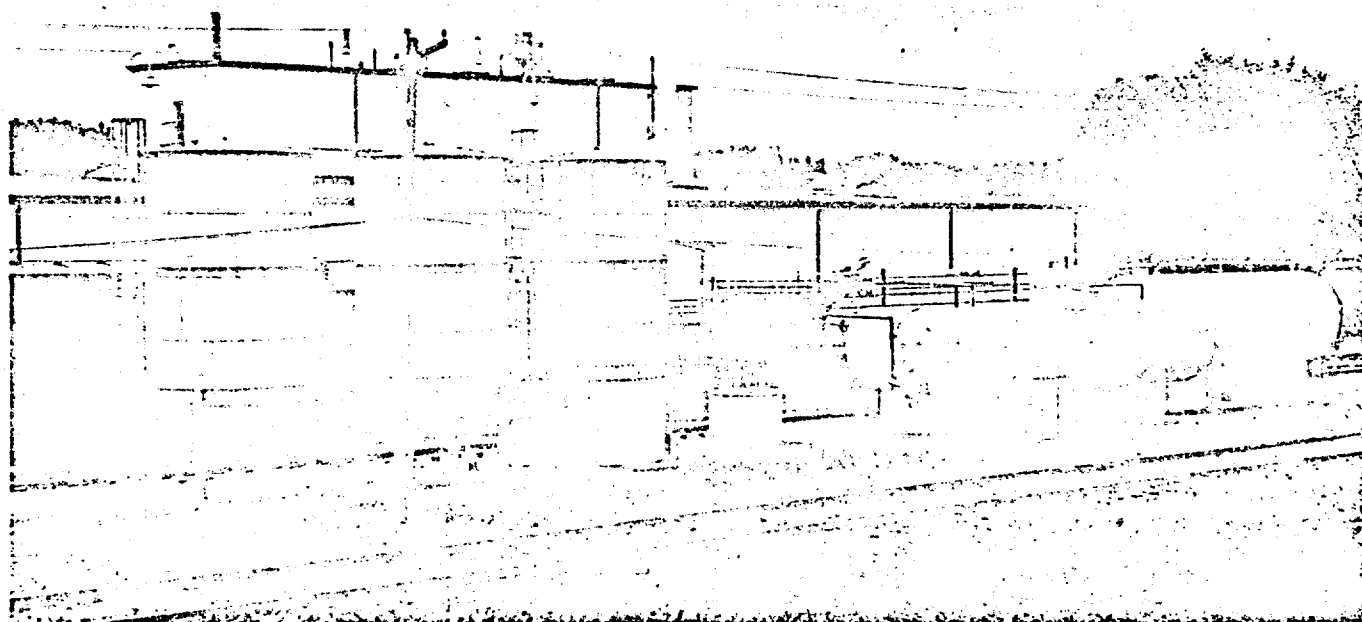
Tank Car Shipments are ordered by more and more concerns. Shown is double-track, tank-car loading complex. The cars on either track are filled and on their way within three hours. **Tank trucks** are quickly filled at a specially designed station on the opposite side of plant.

Preparing Versatile Sugar Color
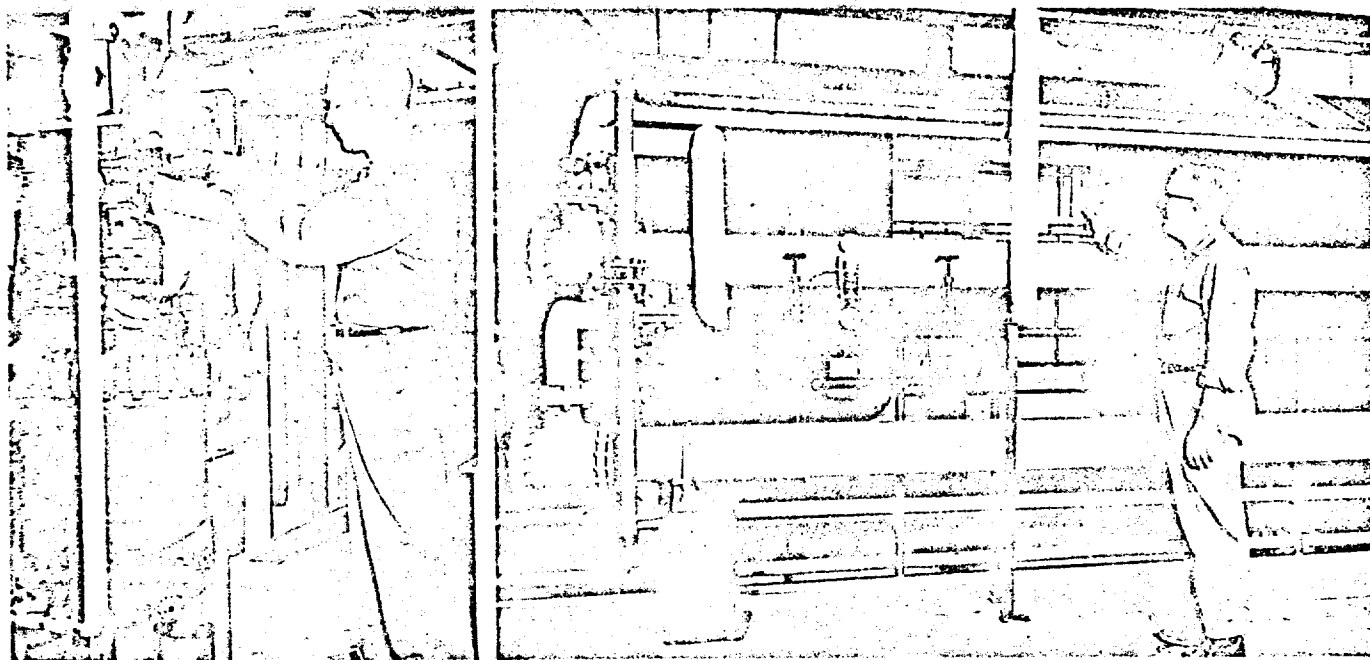
Another Sethness service is the blending of caramel colors for special uses. Individual blends are carefully developed in this unique stainless steel equipment by caramel color experts. *Pair of heat exchangers* (upper right) operated in series to cool caramel syrups. Each heat exchanger is 8 in. diam. x 14 ft. long. This process rapidly reduces temperature from 210° to ideal loading/shipping temp. (90°), assuring greater stability, highest quality.

average of 2.9-3.0. Usual method of determining pH is with the glass electrode.

Flavor and aroma of caramel is also a factor. It appears to be made up of two components—a taste due to its acidity and a taste due to its caramel nature. If the caramel is either manufactured or stored in unsuitable equipment, it is apt to carry a metallic taste due to pickup of excessive amounts of iron or other metals. Use of stainless steel equipment entirely eliminates this possibility.

Degree of taste due to acidity depends on the particular type of caramel being considered. Whereas this taste can be modified, the caramel flavor or taste is natural to the product and is unalterable.

However, such modification is seldom necessary because the quantity of caramel used to color a given product is usually so small that it adds no flavor to the finished product. This can also be said for caramels processed in pressure kettles. But one of the characteristics of pressure-kettle colors is a somewhat stronger taste and aroma than those found in caramels processed in open kettles.

Beverage Types

Caramel molecules in an aqueous solution are colloidal. They carry small electrical charges which may be either positive or negative depending on the method and catalysts used in manufacture. Flavoring essences used in most food products, including soft drinks, also contain colloidal material, such as tannin. These particles are negatively charged when in slightly acid products.

Occasionally refined sugar and purified water will also contain small quantities of negatively charged colloidal materials. Thus, if we introduce caramel molecules with positive charges, mutual attraction between oppositely charged molecules causes them to combine and form large electrically neutral particles. These are insoluble, so turbidity or flocculation takes place in the beverage.

Caramel in aqueous solutions, as in soft drinks, must be chemically tailored to be compatible with other ingredients. The chemist's yardstick for this tailoring is the isoelectric point or the pH at which the colloidal charge is electrically neutral.

At pH's above this point, caramel is negatively charged in solution. Below this pH it is positively charged. Therefore, soft drinks need a caramel whose isoelectric point is below the pH of the beverage. Good beverage caramel must carry a strong negative charge and its isoelectric point should be at a pH of 1.5 or less to allow the user a suffi-

Summarizes Latest Theories

Caramel is found to a greater or lesser extent whenever proteins and sugars are cooked together, or when sugars are heated alone.

But so many simultaneous reactions are possible that no one positively can state what chemical reactions actually occur and what the end-products will be.

Generally, it is believed that formation of caramel from dextrose is a type of polymerization. Original dextrose units are built into larger dextrin-like molecules, some containing 36 carbon atoms and others 96 or more. There is loss of water on polymerization.

What direct relationship exists between these complex groups and the intensity of caramel color is not clear. But this article summarizes the latest theories.—*F.W.P.*

to the combination of catalysts and time used.

Storage

After filtration, caramel is stored in large tanks or packaged in plastic-lined drums or barrels. Often, it is filled into tank cars for shipment to larger users.

Carmelization is believed to continue after processing, although at a very slow rate. For that reason, the user should store the caramel at cool temperature. Such storage does not affect a quality caramel, which may stand over a year without thickening. In fact, some grades will keep for as long as five years. In any event, the user should select his source carefully if he contemplates carrying a large inventory.

Storage of caramel in the case of bulk users presents a different problem. They must have facilities large enough to accommodate 90,000-lb. deliveries. And these should be constructed of stainless steel to eliminate metallic contamination.

Suitable railroad sidings are necessary, and adequate steam lines must be available to heat the coils of the tank car during cold weather. This facilitates removal. But extreme care must be taken not to burn the caramel while doing it. For this, steam at 10 psi. maximum should be used to warm the caramel gradually over a period of hours.

On prolonged storage, however, caramel resinifies into an amorphous, irreversible gel which is not usable.

More knowledge of this gelling may lead to a better understanding of the caramelization reaction, since the gel stage seems to be its end result. But the following paragraphs reveal what is known.

Reaction Mechanisms

Considerable research has been conducted on "browning reactions," into which category caramel formation falls. Caramelization is one or a combination of three broad types of browning**:

1. Maillard reaction—comprising carbonyl-amino reactions of aldehydes, ketones, and reducing sugars with amines, amino acids, peptides, or proteins.
2. True caramelization — due to heat alone. Occurs when polyhydroxy carboxylic compounds, such as sugars and polyhydroxy carboxylic acids, are heated to relatively high temperatures in the absence of amino compounds.
3. Oxidative reactions — on food constituents, which may or may not be enzyme catalized.

Some workers believe that reactions (1) and (2) do not require the presence of oxygen to produce browning, while others disagree. Regardless of type, browning is caused by the formation of colored and unsaturated polymers of varying and unproved composition.

It appears to depend on the presence of carbonyl compounds. Reducing sugars can be transformed by dehydrations*** and/or fission to enediols and osones, and to reductones and dehydroreductones which provide alphadicarbonyl or conjugate dicarbonyl groups and color. Or three-carbon chain compounds, such as acetol and pyruvaldehyde, may be formed. These can react and polymerize to form other intermedi-

** Hodge. John E., "Dehydrated Foods. Chemistry of Browning Reactions in Model Systems." *Agricultural & Food Chemistry*, Oct. 14, 1953, p. 928.

*** *Advances in Carbohydrate Chemistry*, Academic Press, N. Y. C., vol. 6, p. 92.

ates under correct conditions of pH and temperature.

Mechanism of browning in sugar-amine systems apparently follows seven steps, with three proposed stages:

A. Initial colorless stage
  1. Sugar-amine condensation
  2. Amadori Rearrangement from normally substituted glucosylamine to the keto-enol compound, normally substituted 1-amino-1-deoxy-2-ketose.

B. Intermediate colorless or yellow stage
  3. Sugar dehydration
  4. Sugar fragmentation
  5. Amino acid degradation

C. Final highly colored stage due to melanoidin formation
  6. Aldol condensation
  7. Aldehyde-amine polymerization; formation of heterocyclic nitrogen compounds.

Reactions (3) and (4) are believed to be of prime importance with (5) secondary.

Formation of caramel color, with the many possible reactions, plus a large number of controlling variables, such as temperature, pH, and concentration of reactants, makes the chemistry of browning an intricate subject. Thus, the exact chemical composition of caramel color is not known.

Burning of sugars to produce caramel color developed as an art, not a science. But research is now making strides to explain the art that has made this valuable product possible.

Above article is based on talk given by the author at a Sectional Meeting of the Institute of Food Technologists in Rochester, N. Y.

ULTRASPECTROSCOPIC ESTIMATION OF COLORING SUBSTANCES IN SUGAR PRODUCTS

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SUMMARY

It was found that, in the near infrared (IR) region of the spectrum, coloring substances in sugar products practically do not absorb light at all, whereas in the visible region of the spectrum their absorption curves are similar and only in the ultraviolet (UV) region (below 320 mμ) do these substances exhibit selective light absorption and differ in the shape of their absorption curves. This fact has made it possible to use the spectral absorption method for the quantitative determination of individual coloring substance present in a mixture and to establish the fact that the basic color of sugar products is due to products of the alkaline breakdown of invert sugar and melanoidins.

The coloring of sugar products is due mainly to products of the alkaline breakdown of invert sugar, caramels, and melanoidins. In order to investigate the quantitative content of these substances in industrial products, we have utilized methods used in absorption spectroscopy.

It is known that during passage of light through a solution a certain absorption of light takes place, which depends on the nature of the dissolved substance and the length of the light wave, λ . Many substances possess a selective light absorption in the UV region of the spectrum (200-380 mμ), in the visible region (380-760 mμ) or in the IR region (above 760 μ). Light absorption obeys Buger's law according to the formula:

$$D = kcl \qquad (1),$$

where D is the optical density, read on the instrument scale,

k is the absorption factor

c is the concentration of the substance, in g/l

l is the thickness of the solution layer, in centimeters.

By the method proposed by M.I. Barabanov [2] and V.A. Smirnov [3] and

slightly modified by us, we obtained invert sugar alkaline decomposition products by heating a glucose solution with asparagine, and caramel by heating refined saccharose in a thermostat at 185-190°C to a 14% weight loss. The first two substances were isolated from their solutions on a chromatographic column by absorption on activated charcoal and desorption with 25% pyridine solution. The latter, after filtration, was evaporated on a boiling water bath under vacuum. To remove traces of pyridine, a small amount of distilled water was added and the mixture evaporated. The coloring matter, dissolved in a small amount of water, was then dehydrated in a vacuum desiccator, and the powder obtained was stored in closed bottles.

In studying saccharose caramels it was found that caramelan was mainly formed under sugar manufacturing conditions; therefore, this product was isolated from the caramel obtained with the aid of ethyl alcohol and further work was done on this product. The coloring substances thus obtained were dissolved in distilled water, were brought up to a definite concentration and pH7, were carefully filtered through a filter equipped with a porous plate No. 4, and were investigated in spectrophotometers SF-4 and IKS-12 in the 205-2500 mμ region.

During this investigation it was found that the coloring substances do not possess a noticeable light absorption in the IR spectral region, whereas in the visible region the absorption curves have the same configuration and differ only in their light absorption intensity. Only in the UV region, below 320 mμ, could a light absorption characteristic for each substance be observed, as shown in the diagram (see p.133 of original article), where results of measurements are shown in the coordinates $\log D = f(\lambda)$.

The configuration of the curves in such coordinates does not depend on the concentration of the substance in solution and on the thickness of the measured layer.

The spectral curve 1 of invert sugar alkaline decomposition products exhibits a steep rise in the range of 360-260 mμ, forming a peak at 255-200 mμ and a minimum at 225-230 mμ, further maintaining its rise into the deep UV region. The caramelan curve 2 has two peaks: a greater one at 282 mμ and a smaller one at 225 mμ, and has two minimums at 245 and 210 mμ. At the same

time, the rise and fall of this curve is sharper than in other coloring substances. Melanoidins (curve 3) form a peak at the wavelength 290-300 mu and a minimum at 265 mu. In the UV region of the spectrum, the above substances differ sharply from each other in their light absorption intensity and can be arranged in the following series: melanoidins $>$ invert sugar alkaline decomposition products $>$ caramelan. This can be clearly seen from Table 1:

A, mu	K_a	K_b	K_c
400	0.70	0.15	5.25
380	0.85	0.20	6.65
360	1.06	0.27	8.65
340	1.35	0.33	11.00
320	1.75	0.61	13.00
300	2.57	4.10	16.70
290	3.25	7.65	17.00
282	4.20	8.65	16.10
270	5.80	6.55	15.00
260	6.60	3.70	14.90
250	6.70	1.84	15.50
240	6.30	1.77	16.50
230	5.95	2.70	18.50
225	5.95	2.90	20.00

showing values of the absorption factors of invert sugar alkaline decomposition products (K_a) as a function of the wavelength λ ; these factors are calculated according to formula (1) for concentrations in grams/liter and a thickness of solution layer of 1 cm.

The difference in the shape of spectral curves and in the intensity of light absorption was utilized by us to evaluate products containing a mixture of these coloring substances, both from a qualitative and (in a rough form)

quantitative aspect. For example, the curve of coloring substances present in molasses from the Ramonski sugar mill, isolated by adsorption chromatography (curve 4 in the diagram), has a shape similar to that of the curve of invert sugar alkaline decomposition products. And, indeed, as will be shown further in this article, a considerable amount of these products is present in coloring substances of molasses.

To obtain data on the quantitative ratio of individual coloring substances found in molasses and other products, we have used an analytical spectroabsorption method [4]. This method is applicalbe when Buger's law is valid from a concentration standpoint and is based on the additivity of the optical densities of components in the mixture, i.e. in case the following equality is valid:

$$D = D_1 + D_2 + \ldots \ldots D_n \qquad (2)$$

(We have established experimentally that solutions of the coloring substances obtained fulfill the above conditions).

Let us assume that coloring substances of the solutions being investigated contain mainly invert sugar alkaline decomposition products, caramelan and melanoidins. Using the curves shown in the diagram, we select for each substance a characteristic wavelength at which an absorption peak is observed and other components exert a minimum effect. Thus, for invert sugar alkaline decomposition products $\lambda_1 = 250$ mμ, for caramalan $\mu_2 = 282$ mμ, and for melanoidins $\lambda_3 = 300$ mμ. After designating respectively the absorption factors of coloring substances at these wavelengths by means of k_a, k_b, k_c and the optical densities of the investigated product by means of D_1, D_2, D_3, measured at λ_1, λ_2, λ_3, we can set up a system of three equations with three unknowns, in which the mixture contains x grams per liter of invert sugar alkaline decomposition products, y grams per liter caramelan and z grams per liter melanoidins:

$$D_1 = xk_{a1} + yk_{b1} + zl_{c1}$$
$$D_2 = xk_{a2} + yk_{b2} + zk_{c2}$$
$$D_3 = xk_{a3} + yk_{b3} + zk_{c3}$$

The system of equations of the first degree can be solved with the aid of determinants with respect to x, y, z. Substituting into the equations thus obtained values of the coefficients given in Table 1, we get:

$$x = \frac{79D_1 + 32.7D_2 - 104.4D_3}{390}$$

$$y = \frac{72D_2 - 28.5D_1 - 43D_3}{390}$$

$$z = \frac{50.25D_3 - 5D_1 - 22.75D_2}{390}$$

After measuring in the investigated product the optical densities D_1, D_2, D_3, referred to a thickness of solution layer of 1cm, and substituting these into equations (4), (5) and (6), we can determine the quantitative ratio of coloring substances. For example, by taking readings with a photometer on molasses at wavelengths of 250, 282 and 300 mu and a layer thickness of 1 cm, optical density values $D_1 = 0.645$, $D_2 = 0.600$, and $D_3 = 0.430$, respectively, are obtained;

then

$$x = \frac{79 \times 0.645 + 32.7 \times 0.600 - 104.4 \times 0.430}{390} = 0.0654 \text{ g/liter}$$

$$y = \frac{72 \times 0.600 - 28.5 \times 0.645 - 43 \times 0.430}{390} = 0.0161 \text{ g/liter}$$

$$z = \frac{50.25 \times 0.430 - 5 \times 0.645 - 22.75 \times 0.600}{390} = 0.0123 \text{ g/liter}$$

Consequently, the total amount of coloring substances present in 1 liter is equal to 0.0938 g/liter, and expressed in percent x = 70%, y = 17%, z = 13%.

the visible region of the spectrum; for this reason, in rough (approximate) calculations, caramelan can be disregarded. In this case, the quantitative determination of the principal coloring substances in sugar products (inver sugar alkaline decomposition products and melanoidins) merely involves the finding of only two unknown quantities, x and z, which makes computations considerably simpler.

In conclusion, it should be noted that although the coloring substances have absorption bands at different spectral locations (λ_1, λ_2, λ_3), except in caramelan, these bands in other substances are blurred and overlap each other. This, and the fact that other products, interacting with light, are also present in sugar solutions, in addition to the coloring substances mentioned above, affects the accuracy of determination of x, y, z. The present method can also be used to estimate more complex mixtures; however, the accuracy of calculations decreases with an increasing number of components.

Conclusions

1. Invert sugar alkaline decomposition products, caramelan, melanoidins and coloring substances of molasses exhibit characteristic absorption curves in the UV region (205-320 mμ), which differ from each other in their shape, This fact makes it possible to estimate qualitatively a mixture of coloring substances on the basis of its absorption curve.

2. Individual coloring substances in the mixture exhibit an additivity of their optical densities.

3. Using the method described above, it is possible to determine the quantitative ratio of coloring substances in sugar products.

4. The basic color of sugar solutions is due to invert sugar alkaline decomposition products and melanoidins.

Results obtained by this method for some products from a sugar mill are listed in Table 2.

Content of coloring substances, %

Coloring substance	Juice before evapora- tion	Sirup		First- strike massecu- ite	Molasses
		After evapora- tion	Before vacuum still		
Invert sugar alkaline decomposition products	94	83	79	78	70
Caramelan	4	14	14	15	17
Melanoidins	2	3	7	7	13

From the results given in Table 2, it is possible to follow the changes in the coloring of the sugar mill products. Thus, from the juice before evaporation down to molasses the relative amount of invert sugar alkaline decomposition products dropped from 94 to 70%, the amount of caramelan increased 4-fold, while that of melanoidins increased 7-fold. Caramelan was formed mainly at the evaporator station. The amount of melanoidins increased in a uniform manner.

If the content of coloring substances in the solutions is estimated on a weight basis, then the major portion (70-80%) consists of invert sugar alkaline decomposition products. This can be explained by the fact that in sugar manufacturing technical processes take place in an alkaline medium at high temperatures. However, if the color intensity of coloring substances is taken into account, then the basic color of sugar products is due to products formed by alkaline decomposition of invert sugar and melanoidins. Caramelan, on the other hand, does not exert a noticeable effect on the color of solutions in

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ИЗВЕСТИЯ ВЫСШИХ УЧЕБНЫХ ЗАВЕДЕНИЯ

УЛЬТРАСПЕКТРОСКОПИЧЕСКАЯ ОЦЕНКА КРАСЯЩИХ ВЕЩЕСТВ САХАРНЫХ ПРОДУКТОВ

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Установлено, что в ближней инфракрасной области спектра красящие вещества сахарных продуктов практически не поглощают свет, в видимой — их абсорбционные кривые схожи между собой и только в ультрафиолете, ниже 320 ммк, обладают селективным светопоглощением и различаются между собой по конфигурации кривых. Это позволило применить спектроабсорбционный метод для количественного определения отдельных красящих веществ в смеси и установить, что основная окраска сахарных продуктов обусловливается продуктами щелочного распада инвертного сахара и меланоидинами.

Цветность сахарных продуктов обусловливают в основном продукты щелочного распада инвертного сахара, карамели, меланоидины. Для изучения их количественного содержания в продуктах производства мы воспользовались методами абсорбционной спектроскопии.

Известно, что при прохождении света через раствор происходит некоторое поглощение света, зависящее от природы растворенного вещества и длины световой волны λ. Многие вещества обладают селективным поглощением света в ультрафиолетовой области спектра (200 — 380 ммк), видимой (380 — 760 ммк) или инфракрасной (выше 760 ммк). Поглощение света подчиняется закону Бугера по формуле [1]:

$$D = kcl, \qquad (1)$$

где D — оптическая плотность, отсчитываемая на шкале прибора;

k — коэффициент поглощения;

c — концентрация вещества, г/л;

l — толщина слоя раствора, см.

По методике, предложенной М. Н. Барабановым [2] и В. А. Смирновым [3] и несколько измененной нами, получены продукты щелочного распада инвертного сахара — нагреванием раствора глюкозы со свежеобожженной известью, меланоидины — нагреванием раствора глюкозы с аспарагином, карамель — при нагревании рафинированной сахарозы в термостате с температурой 185—190°С до потери 14% веса. Выделение первых двух веществ из растворов производили на хроматографической колонке путем адсорбции их активированным углем, а десорбцию — 25%-ным раствором пиридина. Раствор пиридина после фильтрации выпаривали на кипящей водяной бане под вакуумом. Для удаления его следов добавляли небольшое количество дистиллированной воды и выпаривали. Растворенное затем в небольшом количестве воды красящее вещество обезвоживали в вакуум-эксикаторе. Полученный порошок хранили в закрытых склянках.

При исследовании сахарозных карамелей установили, что в условиях сахарного производства образуется в основном карамелан, поэтому с помощью этилового спирта его выделили из полученной карамели и в дальнейшем работали над ним. Полученные красящие вещества растворяли в дистиллированной воде, доводили до определенной весовой концентрации и pH 7, тщательно фильтровали через фильтр со стеклянной пористой пластинкой № 4 и исследовали на спектрофотометрах СФ-4 и ИКС-12 в области 205 — 2500 ммк.

При этом установили, что в инфракрасной области спектра красящие вещества не обладают заметным поглощением света, а в видимой области абсорбционные кривые имеют одинаковую конфигурацию и различаются между собой лишь по интенсивности светопоглощения. Только в ультрафиолете, ниже 320 ммк, обнаруживается характерное для каждого вещества поглощение света, как показано на рисунке, где результаты измерений представлены в координатах lg $D = f(\lambda)$.
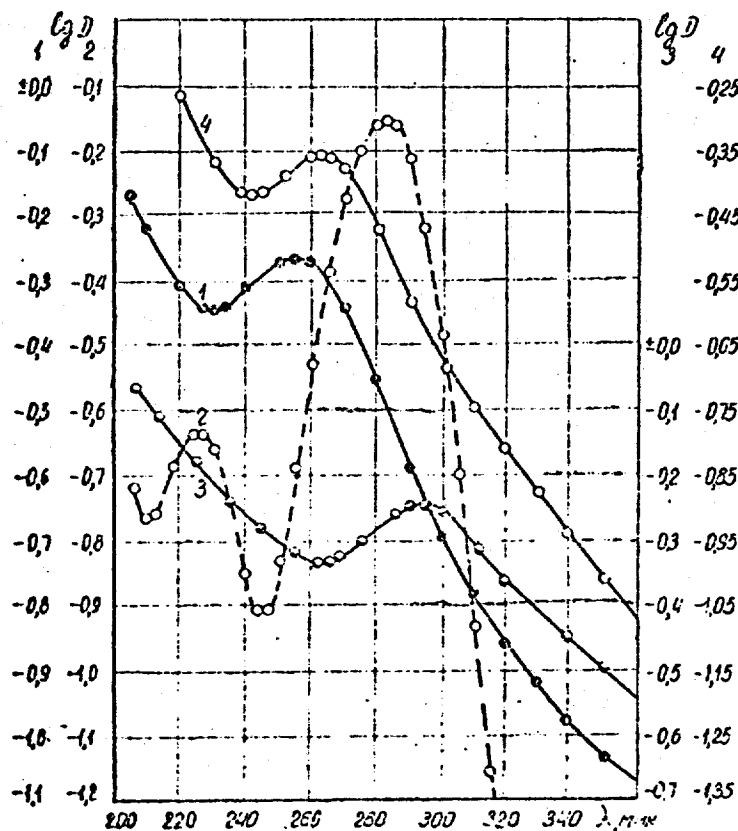
Абсорбционные кривые растворов красящих веществ: *1* — продукты щелочного распада инвертного сахара; *2* — карамелан; *3* — меланоидины; *4* — меласса.

Конфигурация кривых в таких координатах не зависит от концентрации вещества в растворе и толщины измеряемого слоя.

Спектральная кривая *1* продуктов щелочного распада инвертного сахара в пределах 360 — 260 ммк круто поднимается вверх, образуя при 255 — 260 ммк максимум и 225 — 230 ммк минимум, сохраняя в дальнейшем подъем в глубокий ультрафиолет. Кривая карамелана *2* имеет два максимума: больший при 282 ммк, меньший при 225 ммк и минимумы при 245 и 210 ммк. Причем подъем и спад кривой резче, чем у других красящих веществ. Меланоидины (кривая *3*) образуют максимум при длине волны 290 — 300 ммк и минимум при 265 ммк. Указанные вещества в ультрафиолетовой области спектра резко отличаются друг от друга по интенсивности светопоглощения и располагаются в ряд: меланоидины>продукты щелочного распада инвертного сахара>карамелан. Это хорошо видно из табл. 1, где представлены значения коэффициентов поглощения: продуктов щелочного распада инвертного

сахара k_a, карамелана k_b и меланоидинов k_c в зависимости от длины волны λ, вычисленные по формуле (1) для концентрации в $г/л$ и толщины слоя раствора 1 *см.*

Таблица 1

$\lambda,$ м.мк	k_a	k_b	k_c
400	0,70	0.15	5,25
380	0,85	0,20	6.65
360	1,06	0,27	8,65
340	1.35	0,33	11,00
320	1,75	0,61	13,00
300	2,57	4.10	16.70
290	3,25	7,65	17.00
282	4,20	8,65	16,10
270	5.80	6,55	15,00
260	6,60	3.70	14.90
250	6,70	1,84	15,50
240	6,30	1.77	16,50
230	5,95	2.70	18,50
225	5,95	2,90	20,00

Различие в конфигурации спектральных кривых и интенсивности светопоглощения в ультрафиолете мы использовали для оценки продуктов, содержащих смесь этих красящих веществ, как с качественной, так и в приближенной форме с количественной стороны. Например, кривая красящих веществ мелассы Рамонского сахарного завода, выделенных путем адсорбционной хроматографии (на рисунке кривая *4*), по конфигурации напоминает кривую продуктов щелочного распада инвертного сахара. И действительно, как показано далее, в красящих веществах мелассы содержится значительное количество этих продуктов.

Для получения данных о количественном соотношении отдельных красящих веществ в мелассе и других продуктах мы воспользовались аналитическим спектроабсорбционным методом [4]. Он применим при соблюдении закона Бугера в концентрационном отношении и основан на аддитивности оптических плотностей компонентов в смеси, т. е. при соблюдении равенства:

$$D = D_1 + D_2 + \cdots + D_n \tag{2}$$

(экспериментально мы установили, что растворы полученных красящих веществ отвечают указанным условиям).

Допустим, что красящие вещества исследуемых растворов содержат в основном продукты щелочного распада инвертного сахара, карамелан и меланоидины. Используя кривые, показанные на рисунке, выбираем для каждого вещества характерную длину волны, при которой наблюдается максимум поглощения и наименьшее влияние других компонентов. Так, для продуктов щелочного распада инвертного сахара $\lambda_1 = 250$ *ммк*, для карамелана $\lambda_2 = 282$ *ммк* и для меланоидинов $\lambda_3 = 300$ *ммк*. Обозначив соответственно коэффициенты поглощения красящих веществ при этих длинах волн через k_a, k_b, k_c и оптические плотности исследуемого продукта через D_1, D_2, D_3, измеренные при λ_1, λ_2, λ_3, можем составить систему трех уравнений с тремя неизвестными, где смесь содержит x *г/л* продуктов щелочного распада инвертного сахара, y *г/л* карамелана и z *г/л* меланоидинов.

$$\left.\begin{array}{l} D_1 = xk_{a1} + yk_{b1} + zk_{c1} \\ D_2 = xk_{a2} + yk_{b2} + zk_{c2} \\ D_3 = xk_{a3} + yk_{b3} + zk_{c3} \end{array}\right\} \tag{3}$$

Систему уравнений первой степени решаем с помощью определителей относительно x, y, z. Подставляя в полученные уравнения значения коэффициентов из табл. 1, получим:

$$x = \frac{79D_1 + 32,7D_2 - 104,4D_3}{390}, \tag{4}$$

$$y = \frac{72D_2 - 28,5D_1 - 43D_3}{390}, \tag{5}$$

$$z = \frac{50{,}25D_3 - 5D_1 - 22{,}75D_2}{390}.\qquad(6)$$

Измерив в исследуемом продукте оптические плотности D_1, D_2, D_3, отнесенные к толщине слоя раствора 1 *см*, и подставив в уравнения (4), (5), (6), определим количественное соотношение красящих веществ. Например, при фотометрировании мелассы при длинах волн 250, 282, 300 *ммк* и толщине слоя 1 *см* получены величины оптической плотности соответственно $D_1 = 0{,}645$, $D_2 = 0{,}600$, $D_3 = 0{,}430$, тогда

$$x = \frac{79 \cdot 0{,}615 + 32{,}7 \cdot 0{,}600 - 104{,}4 \cdot 0{,}430}{390} = 0{,}0654 \ г/л,$$

$$y = \frac{72 \cdot 0{,}600 - 28{,}5 \cdot 0{,}645 - 43 \cdot 0{,}430}{390} = 0{,}0161 \ г/л,$$

$$z = \frac{50{,}25 \cdot 0{,}430 - 5 \cdot 0{,}645 - 22{,}75 \cdot 0{,}600}{390} = 0{,}0123 \ г/л.$$

Следовательно, всего красящих веществ в 1 *л* содержится 0,0938 *г/л*, а в процентном выражении $x = 70\%$, $y = 17\%$, $z = 13\%$.

Результаты, полученные этим методом для некоторых продуктов сахарного завода, представлены в табл. 2.

По результатам табл. 2 можно проследить за изменением окраски продуктов завода. Так, от сока перед выпаркой до мелассы относительное количество продуктов щелочного распада инвертного сахара уменьшилось с 94 до 70%, карамелана—увеличилось в 4 раза, а меланоидинов—в 7 раз. Карамелан образовался в основном на выпарной станции. Количество меланоидинов увеличивалось более равномерно.

Таблица 2

Красящие вещества	Содержание красящих веществ, %			
	сок перед выпаркой	сироп		утфель-меласса
		после выпарки	перед вакуум-аппаратом	1 продукта
Продукты щелочного распада инвертного сахара	94	83	79	78	70
Карамелан	4	14	14	15	17
Меланоидины	2	3	7	7	13

Если оценивать содержание красящих веществ в растворах по весу, то основная часть (70—80%) приходится на продукты щелочного распада инвертного сахара. Это объясняется тем, что при производстве сахара технологические процессы протекают в щелочной среде при высокой температуре. Но если учесть интенсивность окраски красящих веществ, то основная цветность сахарных продуктов обусловливается продуктами щелочного распада инвертного сахара и меланоидинами. А карамелан в видимой области спектра не оказывает заметного влияния на цветность растворов, поэтому в приближенных расчетах его можно не учитывать. Тогда количественное определение основных красящих веществ сахарных продуктов (продуктов щелочного распада инвертного сахара и меланоидинов) сводится к нахождению лишь двух неизвестных x и z, что значительно упрощает расчеты.

В заключение необходимо отметить, что хотя красящие вещества обладают полосами поглощения при разных спектральных позициях (λ_1, λ_2, λ_3), но, кроме карамелана, у других веществ эти полосы размыты и перекрывают друг друга. Этот факт и то, что в сахарных растворах, кроме учтенных красящих веществ, присутствуют и другие

продукты, взаимодействующие со светом, влияет на точность определения x, y, z.

Настоящий метод пригоден и для оценки более сложных смесей, но с увеличением числа компонентов уменьшается точность вычислений.

ВЫВОДЫ

1. Продукты щелочного распада инвертного сахара, карамелан, меланоидины и красящие вещества мелассы обладают в ультрафиолете (205—320 ммк) характерными абсорбционными кривыми, отличающимися друг от друга по конфигурации, что дает возможность качественно оценивать смесь красящих веществ по ее абсорбционной кривой.

2. Отдельные красящие вещества в смеси обладают аддитивностью оптических плотностей.

3. Применяя описанную методику, можно определять количественное соотношение красящих веществ сахарных продуктов.

4. Основная окраска сахарных растворов обусловливается продуктами щелочного распада инвертного сахара и меланоидинами.

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A STUDY OF CARAMEL COLOR[1]

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Caramel has been used as a coloring material in foods and beverages for many years. The quality of caramels, however, is quite variable, depending upon the material from which they are made, the method of manufacture, and the nature of the products in which they are to be used as a color.

The coloring powers of caramels vary considerably, even though they may be prepared from the same carbohydrate. A given caramel may show stability at one H-ion concentration, and separate from solution at another H-ion concentration. These facts indicate that caramels may be colloidal in nature and that there is a definite isoelectric point for the different varieties of caramel

As early as 1838, Peligot (7) examined the products of the dry distillation of sugars. Gélis (4) in 1858, and Stolle (11) in 1899, also did much to establish the nature of the distillates. Sangiori (10) reported the presence in caramel of furfural, acetone, formaldehyde, formic acid, and acetic acid, all in small quantities. By far the greater portion of the distillate is water.

This leads one to believe that the principal reaction promoted by heating sugars is that of dehydration and that other products are formed to a small extent at a temperature slightly above the melting point of sugar.

Gélis (4) also showed that sucrose, when heated at temperatures around 200°C., was converted progressively into a number of dehydration products by successive dehydration. These products he named in the order of their formation:

caramelan—$C_{24}H_{36}O_{18}$
caramelen—$C_{36}H_{50}O_{25}$
caramelin—

Caramelin was a more highly dehydrated product with a rather uncertain formula but decidedly colloidal.

Graham (5) showed that caramelan and caramelen dialyzed readily and that caramelin did not. Cunningham and Dorée (3) prepared caramelan in nearly pure form by heating sucrose at 170°-180°C. until the loss in weight was 12 per cent. After further purification from alcoholic ammonia they were able to verify the formula of Gélis for caramelan by freezing point methods.

Pictet and Andrianoff (8) working at 10-15 mm. pressure at 185°-190°C., were able to produce the entire series including isosaccharosan, $C_{12}H_{20}O_{10}$. This compound is obtained by simple loss of a molecule of water and is nearly colorless. Further dehydration takes place between molecules, forming larger molecules in multiples of C_{12}.

Ripp (9) prepared caramelan from levulose, proving that sucrose is not the only carbohydrate which will yield caramelan.

Beal and Bowey (1) prepared caramel from glucose with the aid of catalysts such as ammonia, ammonium chloride, sodium carbonate, hydrochloric acid and ammonium sulfate. They showed that with higher temperature and longer heating, caramels of higher coloring power and of less stability were obtained. Beal and Applegate (2) showed that sucrose caramels were equal to or better than glucose caramels.

In order to study caramel more closely and to correlate the colloidal properties with the chemical compounds obtained by previous workers, three series of caramels were prepared from sucrose at temperatures of 190°, 200°, and 210°C. Certain representative caramels from each series were subjected to various H-ion concentrations, to dialysis, and to electrophoresis. The colloidal properties of the compounds of caramel are clearly shown to be related to the temperature and time of heating of the carbohydrate.

PREPARATION OF CARAMELS

A number of caramels were prepared at different temperatures and with varying intervals of time of heating, without the aid of a catalyst. In order to make a systematic comparison of caramels prepared at a given temperature, the loss in weight during a definite interval of heating was taken as a criterion. The small amounts of substances other than water evolved were considered as negligible, and the loss in weight of the sucrose upon heating was considered as water.

Dry sucrose of a high grade was the carbohydrate employed in the experiments. Charges of two hundred grams of sucrose were used. These were weighed upon a trip balance with an accuracy of 0.1 gram.

It was noted in all previous work that little attention was given to accurate temperature control. Since a given charge of sucrose was to be heated at temperatures of 190° to 200°C. over periods of time ranging from 30 minutes to 130 minutes, a wide variation in weights would result in the final products. Obviously it would be nearly impossible to obtain results which could be accurately duplicated. After some preliminary experiments with various methods of heating, this was found to be the case.

An electrically heated device provided with mechanical stirrers was constructed which gave satisfactory results as is indicated in table 1. These runs are typical of the routine runs which are recorded in tables 2, 3 and 4. The duplicate results (b) check the original runs (a) closely, giving rise to an experimental error of about 0.2-0.3 per cent.

TABLE 1. *Influence of time and temperature on loss in weight*

Series No.	Time min.	Temp.	Loss percentage		
			a	b	average
A-1	40	190	0.5	0.4	0.45
A-3	60	190	5.9	5.8	5.85
B-1	30	200	2.3	2.3	2.30
B-2	35	200	4.45	4.45	4.45
B-3	40	200	5.85	6.05	5.95
B-4	45	200	7.85	7.25	7.75
B-5	50	200	8.40	8.25	8.30
C-1	20	210	2.95	2.85	2.90
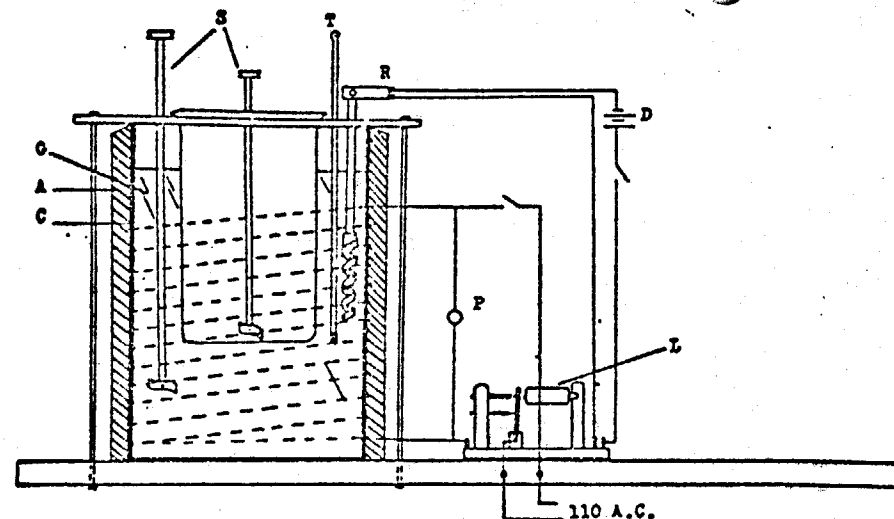
Fig. 1. Design of electric heater for constant temperature bath

DESCRIPTION OF APPARATUS

Figure 1 is a design of the construction of the electric heater. The bath itself is an aluminum cylindrical vessel around which an insulating layer of asbestos paper is closely wrapped. Around the asbestos layer there is placed a coil of twenty turns of No. 18 electric resistance wire. The ends are connected to an electric current. The wire is held in place firmly by a thick layer of fire cement.

A square plate of asbestos board covers the top. Four bolts pass through the corners and into the base upon which the apparatus rests. These bolts serve to hold the bath in a rigid position upon the base. A large hole is cut in the center of the plate of such size that a low-form Griffin Pyrex beaker may be suspended in it and supported only by its rim.

Distributed about the large hole are three small holes. Into one of these is inserted the bath stirrer (S) which keeps the entire bath at uniform temperature by forced circulation. Into another is inserted a thermometer (T) which has been accurately calibrated at the temperature at which the bath is operated. Through the third hole is inserted a regulating device (R) which, by expansion of the metal coil at its lower end, causes the heating current to break when the proper temperature has been reached.

At the top of the regulator are two contact points which, when they touch, close a circuit through the two dry cells (D). This current passes through the magnet on the relay (L). The magnet draws the small vertical arm toward it and causes a break in the 110 volt heating circuit, and the bath ceases to heat. When the temperature of the bath drops sufficiently, the regulator releases the vertical bar, which is pulled over by a small spring and contact is again made in the heating circuit. A pilot light (P), placed across the terminals of the heating unit, indicates when the bath is heating.

The bath medium chosen was glycerin. It becomes very fluid at high temperatures, circulates readily, and has the advantage over oil in that it

can be washed conveniently from the surface of the beaker with water after the completion of a run.

With the device as described, a temperature of 200°C. was evenly maintained over a period of time with a fluctuation of 0.1 to 0.2°C.

METHOD OF PROCEDURE

Into a one-liter beaker, which has been previously described, was carefully weighed two hundred grams of dry sucrose. Both the weight of the empty beaker and of the beaker containing sucrose were recorded. Meanwhile the bath was heated to the desired temperature. The beaker was then placed in the bath, clamped firmly into position, and the time quickly noted.

In a short while the sucrose began to melt and to color slightly. After complete melting had taken place, a slight foaming began to occur which increased rapidly until the foam began to fill the beaker. Rapid stirring at this point with the mechanical stirrer broke the foam sufficiently to keep it well within the beaker. Within a comparatively short time the foaming decreased, indicating that the velocity of the reaction was not so great. As foaming decreased, the color became correspondingly darker. After an interval of time, if the run was carried on for a sufficient period, the foaming ceased, and the mass became quite viscous.

At this point a second stage of foaming began. The mass being very viscous by this time, it was increasingly difficult to stir successfully with the mechanical stirrer. From this point on, stirring by hand became necessary, in order to prevent the very dark viscous mass from rising over the top of the beaker. The velocity of this second reaction slowly decreased and the mass gradually thickened until it was evident that it would be useless to continue the run at the stated temperature.

When the time allotted for the run had expired, the beaker was quickly removed from the bath, the glycerin washed from the outside, and when it had cooled sufficiently, the beaker was weighed. The loss in weight was recorded, as was also the percentage loss in weight.

As soon as one run was completed, a second was immediately started. In this way a series of runs was made at a given temperature, each run being heated a definite number of minutes longer than the previous one.

In this manner, three series of caramels were prepared at temperatures of 190°, 200°, and 210°C. By plotting the time of heating against the percentage loss in weight a number of interesting observations were made and conclusions drawn. Tables 2, 3 and 4 represent the essential data collected for the production of the curves in figure 2.

Fig. 2. Curves showing loss in weight during lapse of time of heating

TABLE 3. *Series B. Caramels prepared at bath temperature 200°C.*

Run no.	Time min.	Loss p'c't'g.	Run no.	Time min.	Loss p'c't'g.
1	30	2.3	11	80	12.1
2	35	4.45	12	85	13.65
3	40	5.9	13	90	14.15
4	45	7.55	14	95	14.25
5	50	8.3	15	100	14.50
6	55	8.9	16	105	14.9
7	60	9.6	17	110	15.4
8	65	10.2	18	115	15.65
9	70	11.3	19	120	15.75
10	75	11.9			

TABLE 2. *Series A. Caramels prepared at bath temperature 190°C.*

Run no.	Time min.	Loss p'c't'g.	Run no.	Time min.	Loss p'c't'g.
1	40	00.45	7	100	09.4
2	50	3.05	8	110	10.45
3	60	5.85	9	120	10.6
4	70	7.2	10	130	11.3
5	80	8.45	11	140	12.05
6	90	8.7	12	150	11.95

TABLE 4. *Series C. Caramels prepared at bath temperature 210°C.*

Run no.	Time min.	Loss p'c't'g.	Run no.	Time min.	Loss p'c't'g.
1	20	2.9	7	50	13.65
2	25	6.85	8	55	14.4
3	30	9.4	9	60	15.15
4	35	10.3	10	65	16.3
5	40	11.5	11	70	16.65
6	45	12.85			

SERIES A. CARAMELS PREPARED AT 190°C.

In order to clarify the description of the general procedure, it is necessary at this point to discuss in detail the production of the caramels at each of the temperatures mentioned.

For each successive run at 190°, the time of heating was extended by ten minutes. The temperature was kept constant at all times to within 0.1° of 190°. At approximately 30 minutes from the time the beaker was placed in the bath, the sugar had completely melted.

There was no foaming until 40 minutes had elapsed. Suddenly foaming started. By the time 80 minutes had passed, the foaming had nearly ceased and the sucrose was merely a dark pasty mass which could be stirred only with difficulty. The color after melting was light brown, but as foaming continued the brown became more intense until, at 80 minutes, it was quite dark.

When 80 minutes of heating had elapsed, the second stage of foaming began. The vapors became more acrid and penetrating to the nostrils. The second stage of foaming was not so violent as the first, indicating that dehydrating was not proceeding so rapidly as before.

The color now became very dark, nearly black; the mass became so viscous that it had to be stirred by hand. From the color it was evident that, with prolonged heating, decomposition products other than water were forming in increasing quantity. After 140 minutes had passed it was considered impractical to continue the run longer.

The above description is that of the longest run made at 190°. All of the shorter runs behaved in an exactly similar manner up to the time allotted to them, when they were removed from the bath.

SERIES B. CARAMELS PREPARED AT 200°C.

At this higher temperature it was to be expected that the sucrose would melt more rapidly, and that the time of heating would be materially shortened in order to form the same caramels which had been produced in series A.

This was found to be the case. The sucrose became completely melted in about 20 minutes and foaming began immediately. The second stage of foaming began in 35 minutes. The third occurred at 55 minutes. A caramel of series B produces much darker solutions than one of series A which had been prepared with the same time of heating.

SERIES C. CARAMELS PREPARED AT 210°C.

The same general observations were made as in series A and B. However, as was to be expected, complete melting had occurred within 15 minutes, and foaming began at once. The second foaming stage began in 19 minutes and the third in 30 minutes.

The data for these three series are recorded in tables 2, 3 and 4. These data were plotted on coordinate paper (fig. 2). With the aid of the curves important deductions were made which are not obvious from the data. In the graph (fig. 1) the loss in weight of the members of each series is plotted against the time of heating in minutes. Three dotted horizontal lines located respectively at 5.23 per cent, 10.52 per cent and 14.03 per cent are included to indicate the points at which isosaccharosan, caramelan, and caramelen should be formed.

In order to prepare isosaccharose ($C_{12}H_{20}O_{10}$), one molecule of sucrose must lose one molecule of water. The weight of water lost would be 5.23 per cent of the molecular weight of sucrose.

The formation of caramelan from sucrose requires the loss of four molecules of water from two molecules of sucrose. The loss in weight required is 10.52 per cent.

Caramelan is formed from three molecules of sucrose by the loss of eight molecules of water. In this case the loss is 14.03 per cent.

These values are theoretical and cannot be attained practically for the reason that other products are formed at the same time to a small extent. Therefore, in order to prepare the purest caramelan that is possible, it would be necessary to heat sucrose until the loss in weight was somewhat over 10.52 per cent. Cunningham and Dorée (3) obtained almost pure caramelan by heating sucrose until the loss was 12 per cent.

Foaming of the caramel as it is heated is indicative of the chemical reaction whereby water is lost. Excessive foaming indicates rapid loss of water, whereas, little foaming indicates little loss. The greatest foaming occurs at the beginning of a definite chemical reaction and at the end there is practically no foaming.

Therefore, in the study of the curves, the horizontal portions indicate the end of one definite dehydration reaction. The rising portion immediately following indicates the beginning of a new reaction.

Curve A. The running temperature of 190° was selected as being the lowest practical temperature at which caramel could be prepared within a reasonable length of time. The melting point of sucrose is approximately 10° below this.

The first stage of foaming started within 40 minutes and had become essentially complete at the 80 minute interval. No decided break can be seen in this portion of the curve. A break should appear in the region of 5 per cent loss, but the relatively low temperature causes the merging of the two reactions into practically one. No foaming stage was noted again until the 80 minute interval was reached. Here a decided rise in the curve is again noticed, showing the completion of the third reaction and the beginning of the fourth.

The first stage should indicate the formation of isosaccharosan, but because of the low temperature, the reaction is not rapid enough to show a distinct break. Consequently, the beginning of the second stage, or the formation of caramelan, is not discernable. The second stage as shown is then really the third stage, and caramelan begins to form within the 80 minute interval.

The loss should be 10.52 per cent, but the curve indicates that caramelan has been formed when the loss is only about 8.5-9 per cent. In order to account for this apparent discrepancy the temperature and viscosity of the caramel must be taken into account. The viscosity is continually increasing while the temperature remains at 190°.

As viscosity increases it becomes more and more difficult to remove the water as rapidly as it is formed, by stirring. Consequently, although the water may be completely liberated, some of it is retained mechanically for a short time before it can be vaporized. According to the curve, when caramelan has been completely formed, there is still mechanically retained as much as 1-1.5 per cent of unvaporized water. The result is that caramelan has been apparently formed with a loss of only about 9 per cent water instead of 10.52 per cent.

At a higher temperature, water would be eliminated more rapidly as it is formed. On curves B and C this is found to be true.

A slight rise in the curve is again noticeable at the 140 minute interval, although no foaming was observed. It was considered impractical to continue this curve further because of the fact that the mass became unstirrable. At higher temperatures the mass remains liquid until the loss is greater.

At the temperature of 190°, the formation of isosaccharosan cannot be detected. The formation of caramelan is readily observed, however, with a loss of weight about one per cent below the theoretical, which has been explained. Caramelan cannot be prepared satisfactorily at this temperature.

Curve B. Foaming begins in about 20 minutes. At the 35 minute interval a slight break occurs which may be construed as the completion of the reaction, sucrose → isosaccharose. The break occurs at the proper point for the formation of isosaccharose, but is not definite, probably because the temperature is high, causing the rapid elimination of water. The second break indicates quite clearly the complete formation of caramelan, and the beginning of formation of caramelen. Here, as in curve A, caramelan is formed with a loss of water about one per cent below the theoretical.

A small break is again observed at the 95 minute interval, although no unusual foaming was observed. The break occurs very close to the 10.52 per cent line, which is theoretical for caramelen. Assuming the mechanical retention of water to be roughly the same, the close agreement between the theoretical loss and the experimental loss can be accounted for by the increase in other decomposition products which vaporize with the water. The higher temperature would favor the formation of substances other than water.

This break shows quite clearly the formation of three dehydration products. Isosaccharosan is formed at the proper place. Caramelan formation is indicated quite distinctly with a loss about one per cent below the theoretical. Caramelen appears to be formed at the proper place also. There is probably mechanical retention of some water, but this is offset by the formation of more decomposition products at the higher temperature.

Curve C. In this case three distinct stages of foaming were observed. The high temperature of 210° caused foaming to occur at 15 minutes and again at 19 minutes. However, water was eliminated so rapidly that it was impossible to record the weights in such a way as to show a break on the curve.

At 30 minutes the third stage began. A distinct break occurs here at the 10.52 per cent line. It is probable that at this high temperature water is driven off almost as rapidly as it is formed and the result is that the loss is almost theoretical for that required to form caramelan.

Another depression is to be noted at the 55 minute interval, but no specific foaming was noted at this stage. This depression is indicative of the complete formation of caramelen.

A consideration of all three curves brings out the following observations:

1. Curve B only, indicates the formation of isosaccharosan.

2. All three curves show clearly the formation of caramelan at or near the theoretical point.

3. Curves B and C show evidence of the formation of caramelen. Curve A cannot be carried far enough to show a similar point.

4. Variations from the theoretical losses can be explained on a basis of mechanical retention of water due to relative temperature and viscosity.

STUDY OF COLLOIDAL PROPERTIES OF CARAMELS

In order to study the colloidal properties, certain caramels were selected from each series of runs which most closely represented the compounds isosaccharosan, caramelan, and caramelen. The caramels selected were as follows:

1. Caramels on curve A which had been formed by heating 70 and 110 minutes, respectively. These will be designated hereafter as A-70 and A-110.

2. Caramels on curve B—B-45 and B-85.

3. Caramels on curve C—C-30, C-60, and C-70. C-70 was selected because it had undergone the greatest loss at the hightest temperature employed, and therefore represented the most highly caramelized product obtained.

Dialysis. No attempt was made to establish any quantitative relationships in the experiments on dialysis. Collodion membranes were prepared by allowing a film to dry upon the inner surface of a large test tube. These were easily removed from the tube by soaking in water.

Solutions of the selected caramels were made by dissolving a small quantity of the caramel in distilled water until the color was an intense brown. The solutions were still dilute with respect to the quantity of caramel present.

Each of the seven selected caramels were placed in separate collodion bags. Each bag was filled and then suspended in a two-liter beaker containing distilled water. Diffusion began immediately as indicated by the color of the distilled water in the beaker. The water was changed every two hours.

Within 6 hours solutions of A-110, B-85, and C-60 had ceased to dialyze. The solutions inside the bags were almost as dark in color as they originally had been. Apparently most of the caramel in each of these bags did not diffuse through the membrane.

After 24 hours, A-70, B-45, and C-30 had practically ceased diffusing. By the color of the contents of the bags, it was concluded that there was some colloidal material present. However, most of the contents were crystalloid because so much time was required to remove it by dialysis.

In the higher portions of the curves the caramels are shown to be quite colloidal in nature, while the lower caramels are shown to be mostly crystalloidal.

Electrophoresis. According to Holmes (6) the charge of electricity on a colloidal particle in suspension is caused by the preferential absorption of positive or of negative ions from the solution on the surface of the particle. A particle thus charged, on electrolysis, will move toward the electrode of opposite sign. Caramels are no exception to this rule.

To demonstrate the mobility of caramel on electrolysis, an apparatus similar to that described by Holmes (6) was constructed. A layer of clear distilled water was carefully superimposed on the caramel solution, the platinum electrodes were immersed in the water layer, and a direct current was passed through at 110 volts for 60 minutes.

The distance which the colored layer at the negative electrode moved downward was carefully measured and noted in table 5.

TABLE 5. *The downward movement of the layer at the negative electrode*

Caramel sol.	mm. lowering	Caramel sol.	mm. lowering
A-70	1.70	A-110	3.55
B-45	0.85	B-85	1.40
C-30	0.40	C-60	5.10
		C-70	1.00

Caramels on the lower portions of the curves (A-70, B-45, and C-30) undergo electrophoresis to a less extent than the upper members. This fact would indicate that the charge upon the lower members is not so great as that on the higher members. The lower caramels would be considered either as particles which are near their isoelectric point, or as particles which border on true solution particles in size. The results of dialysis favor the idea that the particles are near in size to particles in true solution.

Effect of H-ion Concentration. From the results of dialysis and cataphoresis it is concluded that the caramels which have been prepared are electro-negatively charged colloids. Whatever the source of the charge upon the particles, whether it is merely a difference of potential set up between the particles and the medium, or whether it is a case of preferential absorption of hydroxyl ions, the addition of ions bearing opposite charges should have a neutralizing effect upon the charges already present on the particles.

Assuming that hydroxyl ions are absorbed to a greater extent than hydrogen ions, the colloid becomes negatively charged. Addition of acids, or in other words, addition of hydrogen ions, would tend to neutralize the negative charges and eventually cause the particle to become electrically neutral. At the neutral zone coagulation and precipitation of the particles usually occur and the isoelectric point is said to have been reached.

Acids which are highly ionized would be expected to have a greater precipitating effect upon caramel than slightly ionized acids of equivalent strengths. In order to avoid incorrect conclusions, it would be best to choose acid solutions which contained equal molar concentrations of hydrogen ions from different acids.

For the purposes of the experiment, the acids citric, phosphoric and sulfuric were selected. One of these acids represents the weak organic acids which are used in acid beverages, and one a weak inorganic acid (phosphoric), and one the moderately strong inorganic acid (sulfuric).

In order to secure comparative results as far as H-ion concentrations were concerned, solutions of each acid were prepared having the same approximate pH, namely, 3, 2, and 1.5. These solutions were accurately standardized by the electrometric method, using the hydrogen electrode. Table 6 is designed to give the accurate pH of the solutions mentioned in the foregoing. For the purposes of discussion, the approximate values will be used.

TABLE 6. *The pH of acids*

Approx. pH	Citric acid	Phosphoric acid	Sulfuric acid
3			2.90
2	1.97	2.00	2.17
1.5	1.46	1.49	1.56

Since a rise in temperature causes a change in the pH, increasing the degree of ionization of the acid, the effect of both the hot and cold acids on the caramels was determined. The procedure is as follows:

To 50cc. of citric acid, pH-1.5, was added a concentrated solution of caramel until a very deep but still transparent color was obtained. Only a few drops of the caramel solution were required to produce the proper color, and the change in pH by the addition of so small a quantity was considered negligible. The flask was then corked carefully to exclude dust and to prevent evaporation, and set aside in diffused daylight for observation.

The limit of time set for observation was two weeks. If precipitation did not occur within that time the caramel was considered stable toward citric acid of pH 1-5. This procedure was carried out in the same way for the other acid solutions previously described—seven in all. This test constitutes the "cold test" of the different acids on the seven caramels which had been selected for examination of their colloidal behavior.

The "hot test" was conducted in a somewhat similar way. The caramel was added to the acid solution in a small flask. The solution was then boiled gently for a period of twenty minutes, tightly stoppered while still boiling hot, and set aside for observation.

In this way the effect of each acid upon each caramel was determined. The results of these tests are recorded as follows:

TABLE 7. *Effect of cold acids on caramels*

Series no.	Approx. comp.	Time required for caramel to ppt.					
		Citric 2	pH. 1.5	Phosphoric 2	pH. 1.5	Sulfuric 2	pH. 1.5
A-70	Isosaccharosan	—	—	—	—	—	—
B-45	,,	—	—	—	—	—	—
C-30	,,	—	—	—	—	—	—
A-110	Caramelan	—	—	—	—	—	11d
B-85	,,	—	—	—	—	—	11d
C-60	,,	—	—	—	6d	—	75m
C-70	Caramelen	—	—	6d	1m	6d	1m

d = days, h = hours, m = minutes.
— indicates no apparent precipitate found within two weeks.

TABLE 8. *Effect of hot acids on caramels*

Series no.	Approx. comp.	Time required for caramel to ppt.						
		Citric 2	pH. 1.5	Phosphoric 2	1.5	pH. 3	Sulfuric 2	pH. 1.5
A-70	Isosaccharosan	—	—	—	—	—	—	6d
B-45	,,	—	—	—	—	—	—	6d
C-30	,,	—	—	—	—	—	—	3h
A-110	Caramelan	—	—	—	6d	—	—	60m
B-85	,,	—	—	—	?	—	—	75h
C-60	,,	—	—	—	6d	—	—	60m
C-70	,,	—	—	—	1m	—	—	30m

d = days, h = hours, m = minutes.
— indicates no apparent precipitate found within two weeks.

Considering the tables given it is found that cold citric acid, phosphoric and sulfuric acids have no precipitating effect upon the caramels A-70, B-45 and C-30 which represent isosaccharosan on the curves (fig. 1). in two weeks time. From the results of dialysis and electrophoresis, caramels in this region have been shown to be only partly colloidal.

These caramels were found to dialyze much more than the higher caramels and also migrated a shorter distance when subjected to the action of the electric current. When subjected to acid solutions ranging in pH from 1.5 to 2 and 3, there was no precipitation in the cold.

Sulfuric acid with pH 1.5 when hot caused precipitation after an interval of six days had elapsed. In the case of C-30, precipitation occurred much sooner, because C-30 represents a caramel formed by a slightly greater loss of weight, and therefore, is somewhat more colloidal in nature than A-70 or B-45.

The caramels A-110, B-85 and C-60, which represent caramelan on the curves, are not affected by citric either hot or cold. However, cold phosphoric acid (pH 1.5) affects C-60 while hot phosphoric (pH 1.5) affects all the caramels. Sulfuric acids with pH2 and 9 do not affect these caramels when either hot or cold.

C-70, representing caramelen. is not affected by citric acid, but phosphoric (pH 1.5) and sulfuric (pH 1.5) both precipitate it quickly.

The two mineral acids. phosphoric and sulfuric, are more effective precipitating agents than the organic citric acid. When the mineral acids are hot they are more effective than when cold.

SUMMARY

1. Three series of caramels have been prepared, all of which, when loss of weight is plotted against time of heating, show the same general regions in which definite compounds form. These regions correspond in general to the requirements for formation of isosaccharosan, caramelan and caramelen.

2. Caramels of low molecular weight are shown possess low color value, and to dialyze largely. The higher caramels are shown to have opposite properties and are clearly colloidal. All caramels behave as electronegative colloids under electrophoresis.

3. The effect of H-ion concentrations may be summarized as follows:

   a. The caramels do not precipitate in citric acid solutions having pH 2 and 1.5 within two weeks.

   b. The lower caramels are stable to cold sulfuric acid at pH of 1.5, but are precipitated by the same acid when hot.

   c. Phosphoric acid, pH 2 and sulfuric acid, pH 3 and 2 have no effect either hot or cold.

   d. The caramels are most stable in the presence of citrate ions and less stable in the presence of sulfate ions. Phosphate ions have intermediate precipitating action.

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Separation of Caramel Color from Salts and Sugar by Gel Filtration

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Ion exchange was unsatisfactory in isolating the colorants of maple sirup because it failed to separate the colorant from mineral components. A gel filtration method, using cross-linked dextran, was developed and tested in this laboratory. Acid proof caramel colorant could be completely separated from ash salts and sucrose in one pass through a Sephadex column.

As with many foods, the color of maple sirup is an important factor in establishing its grade, quality, and price. In the investigation of the colorants of maple sirup it was necessary to obtain the color isolates. Ion exchange resins (5, 8) were unsatisfactory since the prescribed methods of elution introduced appreciable amounts of salts, and leaching of colored materials from the ion exchange resin introduced contaminants. In addition, some colorants were found to be bound irreversibly on the resin (3), and amino groups of anion exchangers can undergo oxidation or a Maillard-type reaction with carbonyl groups (7). While the ion-exclusion method permitted separation of the colorant of dark molasses from sucrose, it failed to separate the colorant from the mineral components (1). Gel filtration, using cross-linked dextran, has been developed for separating molecular species on the basis of their size (4).

This paper describes a method of gel filtration which permits the separation of relatively high amounts of caramel colorants from sugar and salts.

Experimental

To check effectiveness of color separation by this method, three solutions were prepared and labeled A, B, and C. Solution A, containing only commercial caramel color[1] and

[1] Twitchell Company, Camden, N.J., acid-proof grade. Mention of company and trade names does not imply endorsement by the Department over others not named.

water, was prepared by adding caramel color dropwise to distilled water until a visual match was obtained with the color standard for medium amber maple sirup (138 MacAdam chromaticity units) (2). This solution served as a control in the flame photometric determination of the ash constituents as described below. Solution B was prepared by dissolving 2640 g of sucrose in 1360 ml of water which contained 8.0 g each of potassium malate, sodium acetate, and calcium acetate to approximate the concentrations of organic acid salts in maple sirup. Caramel color was then added to obtain a visual match with Solution A, using a Duboscq colorimeter. This solution was used to determine the effectiveness of gel filtration in the separation of the colorant. Solution C, containing 6% sodium chloride, was prepared in the same manner as Solution B by substituting a 3N sodium chloride solution for the organic salts. This was used for checking effectiveness of the separation of colorant from solutions having a very high ratio of salt to colorant.

To reduce viscosity, 150 ml portions of the above sirup solutions and the control solution were diluted to 200 ml before being added to the columns of dextran gel. The dextran columns were prepared by slurrying 475 g of the dextran gel (Sephadex G-25, 50–270 mesh, manufactured by Pharmacia, Uppsala, Sweden) with water, and transferring the slurry to a 120 × 5 cm i. d. chromatographic column, allowing the excess water to drain to the top of the Sephadex. The dilute solutions of A, B, and C were washed through their respective columns with 3 L of distilled water at approximately 12 ml per min., using a 1 ft hydrostatic head, and the eluate was collected in 20 ml fractions.

Each fraction was analyzed for sugar content by the anthrone reaction (6). The concentrations of potassium, calcium, and sodium in the fractions from Solution B were determined by flame photometry. The corresponding fractions from Solution A, the commercial colorant which contained sodium, were used for reference in the sodium determinations. The fraction of eluate obtained from Solution C (high concentration of sodium chloride)

containing the first trace of the salt was detected by precipitation of the chloride ion with silver nitrate. The color of all individual fractions was estimated by visual comparison with MacAdams chromaticity standards.

To determine recovery of caramel colorant from the dextran column, 150 ml of Solution B was passed through a Sephadex column. All of the colored eluate fractions were combined, yielding 560 ml of solution, and the color was compared to 150 ml of Solution B diluted to the same volume, using a Duboscq colorimeter. The dry weight method was not used for determining colorant because of the unknown composition of the caramel and the danger of causing degradation of the caramel on drying. Absorption spectroscopy was not used because absorption at one wavelength would not necessarily detect the removal of one or more color compounds by the Sephadex.

Results and Conclusions

By using the gel filtration method, it was possible to completely separate acid proof caramel colorant from ash salts and sucrose in one pass through a Sephadex column. A large column of Sephadex (120 × 5 cm i. d.) had to be used since smaller columns, 20 × 2 cm i. d., did not completely separate the colorant after two or more passes. The amount of colorant in MacAdams chromaticity units and amounts of cations (calcium, potassium, and sodium) in the eluate are shown in Fig. 1. The combined weights of the three cations of organic salts were used since they were all eluted in the same fractions. Colorant was completely separated from the organic salts, sucrose, and sodium chloride, as no traces of these materials were found in the colored eluates.

As indicated in Fig. 1, the first 680 ml of eluate was colorless. Apparently the dextran did not retain any colorant. The next 560 ml of eluate contained all of the colorant added to the column, most of it in the first 300 ml. The cations first appeared in the eluate fraction immediately following the elution of the colorants (1240 ml). The sugar and the chloride ion appeared later.

These results indicate that gel filtration provides a simple technique for isolating the higher molecular weight caramel colorant from solutions of sugars and salts.
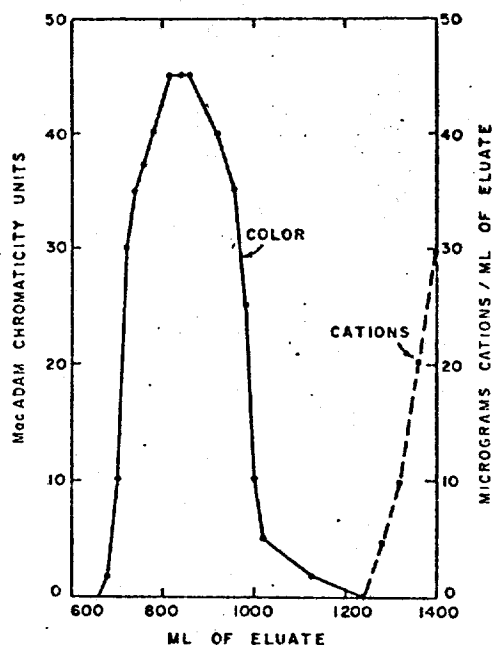
Fig. 1—Separation of caramel colorant by gel filtration from solution containing salts and sugar.

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BIBLIOGRAPHICAL STUDY OF CARAMEL (*)

By Messrs. R. Truhaut, G. Vitte and V.Lassalle-Saint-Jean

The authors report on the various practical and theoretical chemical reactions giving rise to the formation of dark coloring substances of the "Caramel" type. Two basic reactions of the browning of food substances have retained their attention: the pyrogenation of the carbon hydrates and Maillard's reaction. They explain in each case the chemical dynamics and the nature of the polymerization products obtained, either in general or in particular cases.

Historians agree to fix the date of the introduction of sugar cane into Europe and its cultivation in Sicily at the time of the Crusades (circa 1230).

The products extracted from sugar cane were known in Bengala and the Middle East since very ancient times.

The Romans used to give them to their athletes. However, it was not until around the sixth century A.D. that a true trade developed in this field, remaining exclusively in the hands of the Italian Republics for a long time.

Arab caravans used to transport this commodity. Its value was connected to its coloration. The more colored the merchandise the less appreciated. The latter quality was known as "curat nilh", which in Arabic means: sweet pack. It is assumed that this is the origin of the name caramel which is used to designate the dark brown product resulting from the pyrogenation of sugars.

In the sugar industry, caramel is present in a more or less large proportion in all products manufactured; it is found, depending on the method of preparation, in a proportion from 0.4 to 0.5% (1) in sugar. As its presence constitutes a loss, research on the part of specialized technicians is aimed at tracking down and controlling its formation.

While the presence of a high proportion of caramel is found only on exceptional occasions in the sugar industry, the danger of caramelization constitutes a constant worry in the glucose industry, because the glucose is generally obtained by letting a strong diluted acid act on starch.

As fructose is much more sensitive to thermal reaction, the caramel is, also in this case, responsible for any loss and pollution, and these are watched very closely.

However, above anything else, the caramel is a coloring substance. It is defined as a coloring substance of natural origin in appendage 1 of the Official Decree of June 18, 1958: "Coloring substance obtained by heating sugar to a temperature higher than its melting point, but without letting it caramelize until it becomes a brown or black compact mass."

Its taste is generally bitter, this bitterness being increased by the addition of small amounts of alkali or alkaline carbonates introduced during the preparation.

It presents itself as a viscous liquid whose color varies from dark brown to black, having a characteristic odor.

The American Pharmacopoeia (2) lists the following requirements for the caramel:

-- density equal to or above 1.3, taken at 25° C;
-- it is soluble, and its 1% aqueous solution must have a light sepia tint which must be held for a minimum period of six hours and must not precipitate when exposed to solar light during the same period of time.
This caramel is soluble in water, in any proportion.
-- it is also soluble in alcohol of less than 55°, but insoluble in the majority of organic solvents, such as ethyl ether, petroleum, benzine, chloroform, acetone, etc.

As will be pointed out later, caramel is a body mixture. Prepared from saccharose onwards, it is completely soluble in water. However, if the sugar is not refined, insoluble compounds will appear. The amount of these compounds increases with the dehydration.

This caramel is more soluble in alcohols than the one prepared from glucose onwards, due to the presence of dextrine in the latter.

The addition of alkaline carbonates during the boiling increases the coloring power and favors the solubility of certain fractions which otherwise would be insoluble. Solid or pulverized caramels are also used. Solid caramel is generally brownish red, breakable, amorphous and very deliquescent.

CHEMICAL COMPOSITION OF CARAMEL

Scientific concepts and studies pertaining to the chemical composition of the caramel have evolved considerably from the time research started to this day.

As a single chronological report could lead the inexperienced reader to regrettable or irksome confusion, we will adopt a presentation divided into three parts:

The first chapter will cover the research work on the origin and nature of the caramel.

The second chapter will deal particularly with the behavior of sugars under the influence of certain physical or chemical factors.

The third one will mention recent theories applying more specifically to the two commercial categories of caramel we have at present.

CHAPTER 1

Research on the origin and nature of the caramel

In 1838, Eugene Peligot (3) discussed "the action of heat on sugars" in an article entitled "Research on the nature and chemical properties of sugars". He noticed the existence of a substance mixed with the sugar which he designated as caramel "in order to avoid the always troublesome coining of a new name" and to which he assigned the formula: C48H36O18.

He pointed out the presence of acetic acid in the condensate arising from the pyrogenation of sugars, specified the acid nature of the product, and mentioned its precipitation through ammoniacal Pb acetate and barite water.

Caramel was practically not mentioned again in the scientific press until March, 1858, at which time A. Gelis (4) published his "Study of caramel and torrefied products".

Gelis pointed out that Voelkel (5) had repeated and confirmed Peligot's experiments and had given the name of Caramelan to the coloring product, assigning to it the formula: $C_{24}H_{13}O_{13}$.

But his original work was "the study of the 'assamare' (from assare: to grill, roast; and from amarus: bitter) in the tars that distill when the sugar is decomposed completely through burning". This 'assamare' term was attributed to Reichenbach (6) and designated a bitter principle that arises every time organic matters are heated until they become brown.

A. Gelis wanted "to go deeper into the matter", inasmuch as Gerhardt (7) had assumed that Voekel's 'assamare' was identical with Mulder's (8) "apoglucic acid". This need led to a study concluding that three basic substances existed in the caramel: Caramelane, Caremelene and Carameline.

1. CARAMELANE - To obtain this body, Gelis treated the caramel resulting from the boiling of saccharose at a temperature of 210-220°, with 84° alcohol. He kept the alcoholic fraction, evaporated the alcohol at a temperature lower than 1200°, did the setting by means of water and let the existing fraction of the -oses ferment in the presence of beer yeast.

After concentration, the Caramelane is precipitated by absolute alcohol. Its formula is established by treatment with lead acetate and lead salts, the formula being $C_{12}H_{16}O_8PbO$. He also obtained a combination with barium.

2. CARAMELENE - The ordinary caramel, dried up with 84° alcohol, is completely devoid of Caramelane and leaves a residue which partially dissolves when treated with cold distilled water. "The dissolved matter is almost pure Caramelane".

Also in this case, a lead or barium combination is used, thanks to which this formula is obtained: $C_{36}H_{48}O_{24}H_2O$.

Note the existence of three lead combinations:
one obtained in acetic acid medium corresponding
to the formula . . . . $C_{36}H_{48}O_{24}$ PbO
a second one, in strong ammoniacal medium. . . . . $C_{36}H_{48}O_{24}$ 4 PbO
and a third one, in weak ammoniacal medium . . . . $C_{36}H_{48}O_{24}$ 6 PbO

3. CARAMELINE - After the two treatments mentioned above, there remains a residue. "It contains only a single body, Carameline, mixed with variable amounts of more or less carbonized sugar, but this Carameline exists in various isometric states."

It exists in these three forms:

-- a first part soluble in cold water;
-- a second one soluble in warm water and 60% alcohol;
-- a third one insoluble but partially soluble in alkalis.

The whole of these products always gives a precipitate, with $BaCl_2$ as well as with Pb acetate, and Gelis felt that the formation of these various products is directly

connected to the saccharose's loss of weight in water. He established the following scale:

For a reduction of:

10% caramelane is obtained $= C_{12}H_{22}O_{11} = C_{12}H_{18}O_9 + 2\ H_2O$;

14% caramelene is obtained $= C_{36}H_{66}O_{33} = C_{36}H_{50}O_{25} + 8\ H_2O$;

25% carameline is obtained $= C_{12}H_{22}O_6 = C_{12}H_{12}O_6 + 5\ H_2O$.

Besides, having subjected to hot nitric acid treatment these products resulting either from saccharose caramel or glucose caramel, Gelis obtained oxalic acid.

In 1862, Thomas Graham (9) published research on Molecular Diffusion Applied to Analysis.

While studying the raw caramel obtained by heating sugar cane to $210/220°$, he confirmed that the dialytic analysis of this substance corroborated Gelis' notes.

Gelis' Caramelane and Caramelene diffuse and the substance remaining on the diaphragm presents a coloring power equal to five times that of the original raw caramel.

This body can be obtained by precipitating it from its aqueous solutions by means of alcohol.

After drying it at low temperature, this soluble caramel can then be heated to $120°$ without ceasing to be soluble, but if a solution of this same caramel is evaporated directly through water bath, the residue obtained is completely insoluble in cold or hot water. This body has the same composition soluble or insoluble.

Graham notes the colloidal nature of the product. He proposes the formula: $C_{24}H_{13}O_{13}$, and adds:

"Gelis' Carameline analysis does not apply in anything to the compound with which we are dealing." (10).

He verifies that this caramel is excessively sensitive to the action of crystalloid reagents. It is precipitated or coagulated by simple traces of mineral acid, alkaline sulfates, ClNa, as well as by alcohol.

An important fact: the presence of sugar or of little dehydrated products neutralizes the action of the crystalloids.

This was also verified by other authors in their research work.

This caramel is, however, precipitated by certain substances of its own family, such as iron peroxide.

He notes that the slowness of diffusion must be connected to the substance he has just described.

The other products that accompany it, which are more diffusible, are however less diffusible than all the varieties of sugar.

Caramel is 600 times less diffusible than ClNa and 200 times less than sugar.

Graham points out the resemblance of caramel, in its insoluble form, to coal; the caramelization could be the beginning of a colloidal process that is completed in a long period of geological eras, becoming coal.

In 1899, Stolle (11) studied, in turn, the pyrogenation of sugars, and arrived at the following conclusions in a series of articles published from 1899 to 1903.

The principal product is Gelis' Caramelane, obtained by loss of 12% of the initial weight when the sugar is heated at $180-190°$.

Up to 190°, the process is effected only through loss of water; above that, there is a release of CO_2 and acetone.

The monoplombic combination precipitates neither in neutral medium nor in weak acetic medium; it precipitates only in alkaline medium, which did not agree with Gelis' conclusions.

He drew the conclusion that since at the time of the analysis of the molasses the clarification had been obtained through lead acetate, the non-precipitated Caramelane could reduce the Fehling' solution; the said reduction wrongly imputed to the sugar being the reason for the error.

He proposed the formula $C_{12}H_{18}O_9$ for this product with which he prepared a tetracetate and a monobenzoate.

Hydrolized by boiling it 18 hours with diluted SO_4H_2, the Caramelane's dark solution becomes light yellow with formation of humic acid flakes for which Stolle proposes the composition: $(C_9H_{11}O_5)^X$, as well as levulinic acid and hexose resembling dextrose, but which he did not identify.

In 1906, Trillat (12) found, while studying the volatile products formed when the sugar is heated, that:

at 200° 0.1 to 0.3% of formaldehyde is obtained;
at 100° traces are apparent after 24 hours;
at 150° it is detected within a few minutes.

This is why he concluded that caramel is really a combination of polymerized products of formaldehyde.

In 1909, Ehrlich (13), while researching, attained the caramelization of sugar "in vacuum". He felt that the coloring substance of pure sugar caramel is a compound, $C_{12}H_{22}O_{11} - 2 H_2O$, which he called "sacchrone".

He obtained this product by heating sugar in a flask immersed in oil at 200°. The residue remaining after extraction of the other compounds through methylic alcohol was dissolved in water, filtered and evaporated until obtaining amorphous dark brown body easily reduced to powder.

This resarch work was used as a basis by other researchers, the method of caramelizing sugar "under vacuum" offering true scientific guarantees.

In 1917, Mary Cunningham and Charles Doree (14) published a "Contribution to the Chemistry of the Caramel", inspired by Gelis' and Stolle's work.

From the beginning of their article, based on the work of Green (15), Willstatter (16), Fenton (17), Fisher (18), Sestini (19) and Bottomley (20), they propose, in place of the formulas established by Gelis for Caramelane, Caramelene and Carameline (which can be reproached for having an arbitrary content of water), the following formulas as the only ones valid:

Caramelane: $6 C_{12}H_{22}O_{11} - 12 H_2O = 6 C_{12}H_{18}O_2$;
Caramelene: $6 C_{12}H_{22}O_{11} - 18 H_2O = 2 C_{36}H_{48}O_{24}$;
Carameline: $6 C_{12}H_{22}O_{11} - 27 H_2O = 3 C_{24}H_{26}O_{13}$.
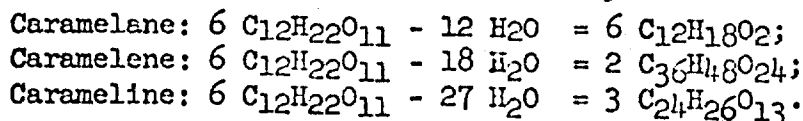
Following Stolle's procedure, they heated the sugar between 180 and 190°:
Caramelane was obtained with 12% weight loss;
Caramelene was obtained with 15% weight loss;
Carameline was obtained with 22% weight loss.

Caramelane obtained through this treatment is a brown, breakable, bitter, odorless and hygroscopic solid that melts at 136° and softens at 100°, soluble in

84° alcohol, pyridine, methylic alcohol, hot crystalized acetic acid, and insoluble in ether, pure alcohol or benzene. It reduces Fehling's solution and silver nitrate.

Aqueous solutions acidified with HCl give a precipitate when resorcine is added, which precipitate is soluble in alcohol and alkalis. Phlorogrucine gives a resembling precipitate, but of a more intense color.

They proceeded next to a series of studies.

1. Caramelane esters - They obtained:

- a tetraacetate;
- a tetrabenzoate;
- a tetranitrate;

- a yellow tetraacetate with a fusion point of 107°, that it insoluble in water or ether, soluble in C_6H_6, warm alcohol or glacial acetic acid and that reduces the Fehling's solution;

- a light tan tetrabenzoate with a fusion point of $105-108^\circ$, soluble in acetone, $CHCl_3$, alcohol, C_6H_6; insoluble in water, ether and light oil;

- a yellow tetranitrate, that is violently inflammable under the influence of heat, and is rapidly soluble in ether or benzene and insoluble in water.

They determined the molecular weights of the Caramelane and its esters.

2. They studied the aldehydic or cetonic nature of Caramelane.

A. With phenylhydrazine:

a). in the presence of acetic acid:

$$2C_{12}H_{18}O_9 + C_6H_5 NH NH_2 = C_{30}H_{38}O_{15}N_2 + 3 H_2O;$$

b). in the presence of SO_4H_2O:
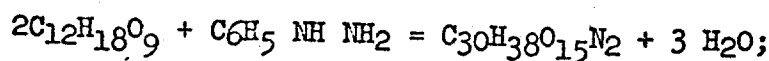
$$2 C_{12}H_{18}O_9 + C_6H_5 NH NH_2 = C_{30}H_{28}O_{10}N_2 + 8 H_2O.$$

B. With semi-carbazide:

$$3 C_{12}H_{18}O_9 + NH_2 NH CONH_2 = C_{37}H_{43}O_{20}N_3 + 8 H_2O.$$
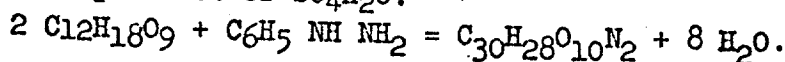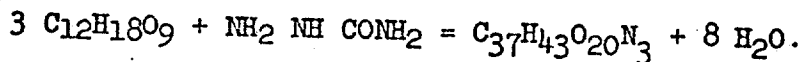
3. Action of acids on Caramelane. - Based on the research work of Conrad et Guthzeit (21), Bottomley (22), Stolle (23), Krober (24), Eller and Tollens (25), Cross (26), Gostling (27), Sestini (28), Willstatter (29) and Fisher (30), they established a series of results indicated in the following chart:

Caramelane

HCl 3% → dextrose methylfurfuraldehyde

HCl 12% → humic acid $C_{24}H_{22}O_{11}$ / nitro derivative $C_{24}H_{23}O_{14}N$

HNO₃ 7% → furfuraldehyde chloro derivative $C_{24}H_{29}O_{18}Cl_3$

HCl 40% → Carameline $C_{24}H_{26}O_{13}$
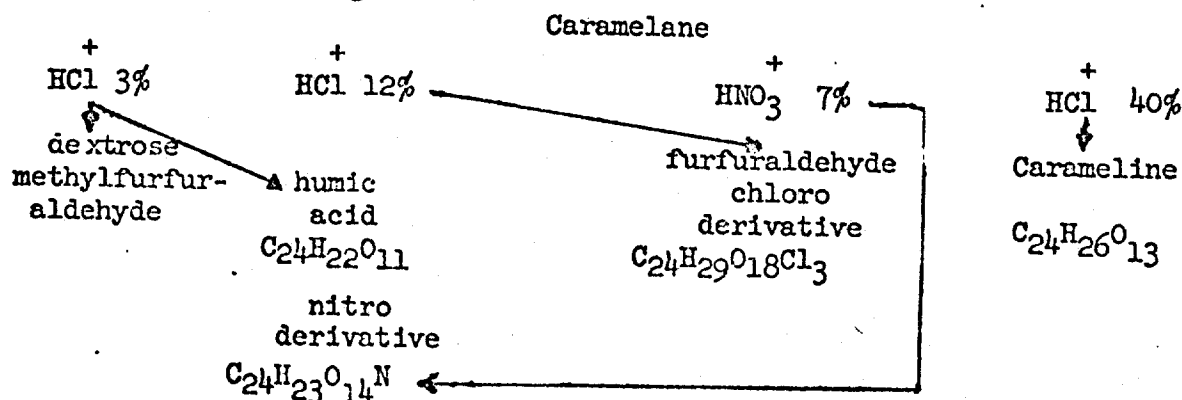
4. Acetolyzation of Caramelane - Referring to the work of Skraup (31) and Born and Nelson (32), the authors concluded that Caramelane is either a monosaccharide or a dissacharide.

5. The action of oxidating agents.-They operated in the presence of 7% NO_3H and 20% bromine and ozone.

a) The action of NO_3H is indicated in the following chart:

$$C_{24}H_{22}O_{11} \qquad C_{23}H_{23}O_{12}NO_2 \qquad + C_{11}H_{15}O_8NO_2$$

humic   insoluble   soluble
acid   acid   acid

$$C_{12}H_{18}O_9$$

$$(C_{23}H_{24}O_{12})$$

$$(C_{23}H_{26}O_{13})$$

isolated by dihydrazone
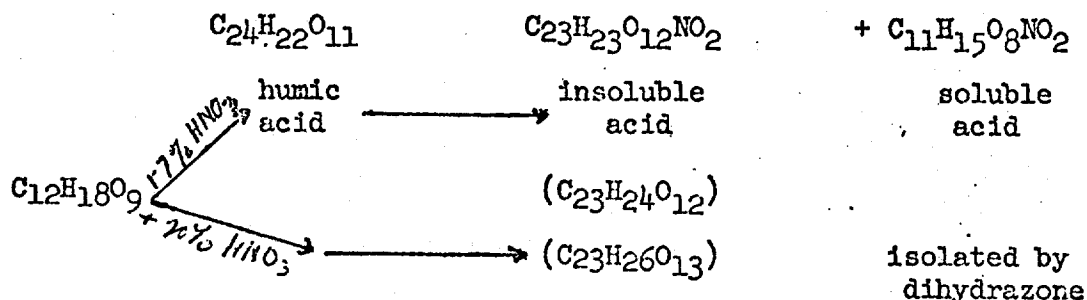
The bromine's action determines the obtaining of the following two products:

$$C_{35}H_{53}O_{29} \; Br \quad \text{and} \quad C_{35}H_{52}O_{29} \; Br_2,$$
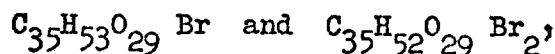
the former requiring 7.9% bromine, and the latter 14.6% bromine.

The authors bring these compounds nearer to the sesquibromo-oxy-sacchulmide obtained by Sestini (33).

With ozone, the authors mark the existence of an unsaturated compound $CH_3CH: CX$.

Ozonide is decomposed by water into $CH_3CHO + X \; COOH$.

Their conclusions are that:
1. Sugar heated to 170-180° loses two molecules of water to give Caramelane $C_{12}H_{18}O_9$, tetraalcohol f.p. 136°, marked by a tetraacetate f.p. 107°, a tetrabenzoate f.p. 105°, and an explosive tetranitrate.

Formula: $C_{12}H_{18}O_9$ or $C_{24}H_{36}O_{18}$.

2. The products formed by Caramelane with phenylhydrazine and semi -carbazide indicate the existence of the group CO and CHO by C_{24}.

3. Treatment through non-oxidating acids gives dehydration and hydrolysis products: dextrose, methilfurfurole, aldehyde, and humic acid.

4. Oxidation gives complex substances.

5. Caramelane is the first step in the process of anhydrous formation and of condensation of the simple sugar to cellulose, humus and Carameline.

Besides, and contrary to Stolle's findings, the authors have found that the molecular weight of Caramelane in water is twice that of the molecular composition, that the tetraacetate gives a double molecular weight in C_6H_6 and that the tetrabenzoate gives a quadruple weight.

In 1924, Pictet (34) also dealt with the caramel problem. He reproached all the other authors preceding him for not having conducted their research on pure products. He based his statement on the fact that the materials analyzed were strongly colored. Their analyses were inaccurate, and their results regarding the fusion points were quite different. He proceeded to a "vacuum" caramelization so that the acid products that come into being in the course of the process be quickly separated. By "vacuum" heating at 180° until a loss of 5% of the weight was attained, he obtained a product which he called isosaccharosane.   This product, dissolved in

methylic alcohol, was precipitated by the addition of acetone and gave an amorphous powder. To this, he gave the formula $C_{12}H_{20}O_{10}$.

Very soluble in water, where it becomes inverted sugar, this product melts at approximately 94°. A hexa-acetate of f.p. $79/80^\circ$ is obtained in the presence of pyridine and anhydrous acetic acid. The reducing power on the Fehling is half of that of glucose. Pictet concluded that by heating beyond the fusion point of the saccharose, the latter first becomes an isomer and then the dehydration takes place. By prolonged "vacuum " heating, Pictet obtained Caramelane of formula $C_{24}H_{36}O_{18}$ f.p. $144/145^\circ$. By prolonging the heating even further, he obtained Caramelene of formula $C_{36}H_{50}O_{25}$ of a f.p. of $104/106^\circ$.

We will also mention Dr. A. Schweitzer's (35) thesis, which constitutes a very complete document of bibliographic synthesis. Reproducing, carrying through and checking the entire research work done on the Caramel, we feel that this is the latest document regarding the traditional criteria of caramel analysis. Dr. Schweitzer thinks that "caramel is a mixture of Pictet's isosaccharosane and humin, the latter being a carbon hydrate in a very elaborate polymerized form". He adds that "humin" is insoluble in water and that in the presence of a certain amount of isosaccharosane the union of these two products becomes soluble".

Doubt and negative answers incited Joszt and Molinski (36) and Cruisheer (37) to further research.

Joszt and Molinski reached the conclusion that neither Caramelane nor Caramelene nor Carameline exists. They studied these three substances in dialysis water. They studied their degree of acidity, their superficial tension, their viscosity and their coloring intensity, which they consider as very close. They feel that there are very small differences in their elementary constitution.

Cruisheer feels not only that all the above-mentioned substances denied by Joszt and Molinski are fictitious, but also that the isosaccharosane does not exist.

Von Elbe (38) considers caramel as a mixture of two products close to saccharose, one of these products being constituted by a colloidal dispersion of humin.

Schweitzer notes "that the acetate of one of the products has a composition corresponding to the formula of a hexa-acetate of isosaccharosane".

A great many authors have mentioned in their publications the composition of the caramelization products.

Janeck (39) considers, for instance, that Sabanejeff's (40) formula

$$11\ C_{12}H_{22}O_{11}\ =\ 7\ CO_2\ +\ 27\ H_2O\ +\ H_{188}O_{80}$$
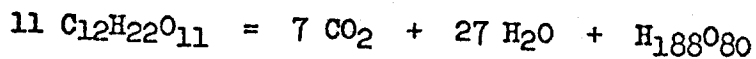
was better than Gelis' formula for the Carameline obtained through dialysis.

Brown and Zervan (41) gave the name of saccharane to the product that Ehrlich called earlier saccharone. "One part of this body colors 10,000 parts of water dark brown, which color can be increased by the addition of alkali. It has less taste and is not precipitated by the under-acetate of lead as the coloring substances of the syrup." As time went by, these tests appeared unreal.

The data acquired then allowed researchers to connect the caramel with the humic by-products resulting from sugar, which will be the subject of our second chapter.

CHAPTER II

Behavior of sugars under the influence of certain chemical or physical factors

Documentation on this is quite abundant. We will deal more particularly with writings that prove interesting for our studies and connected with research on carbon hydrates. Not all the documentation available will be mentioned, because the list of writings relative to humus research is quite long. Indeed, if we take into consideration that humic substances are formed by "decomposition of carbon hydrates under the action of diluted or concentrated acids, by the hydrolysis of albuminoid substances, or by the oxidizing action of alkalis or polyphenols", we can understand why our report is limited.

Furthermore, we will respect to a great extent the chronological order of the writings. However, there will be occasion when we will break this rule in order to meet requirements created by the flow of methods that guided the authors.

We believe it is advisable to familiarize the reader right at the beginning of this report with the terms that will be encountered, particularly those relative to the humic substances.

Everybody agrees to regard Sven Oden's (42) report in Kolloid. Beih. 11, p. 75-260, as a basic element of this classification.

Hoppeseyler (43) having determined the hymatomelanic acid, the humic substances were already being classified in 1889 as humic acid as distinguished by Berzelius (44) and as fulvic acid as distinguished by Oden, the said fulvic acid decomposing into Berzelius' Creen and Apocreen acid. This classification is said to have been completed in 1938 by Springer (45) who made a distinction between brown humic acids and gray humic acids, which, when added to Hoppeseyler's (43) hymatomelanic acid, reconstituted Berzelius' humic acid. We will schematize this whole in the following chart:

Humic substances

Fulvic acid			Humic acid		
Creen Acid	Apocreen Acid		Hymalomelanic Acid	Brown Humic Acid	Gray Humic Acid
Slightly yellow	yellowish brown		brown	Dark brown	grayish black

In 1786, F.K. Achard (46), the creator of the beet industry, obtained humic acid starting from peat, through alkaline extraction and acid precipitation (SO_4H_2).

Dobereiner (47) was the first one to use the term "humic acid".

Braconnot (48) was the first one to obtain, in 1819, artificial humic products beginning from sugar under the action of the acids.

Thomson (49), having treated the peel of Ulmus Nigra, obtained, through alkaline extraction and acid precipitation, a product which he called ulmin. For a certain period of time, the terms humic acid and ulmin were used.

In 1839, Berzelius (44) gave humin the formula $C_{32}H_{32}O_{16}$. By oxidizing peat through NO_3H, he obtained two acid derivatives which he called Creen acid and Apocreen acid.

In 1835 and 1836, Malaguti (50) started the research work that was continued later by other authors.

In 1840, Mulder (51) obtained with sugar, through the action of diluted acids (SO_4H_2, HCl), four bodies, of which two are soluble in alkalis and two are insoluble. Two are maroon: one of them, the ulmic acid, is soluble, its formula being $C_{40}H_{28}O_{12}$; the other, insoluble, is called ulmin, its formula being $C_{40}H_{32}O_{14}$. The other two are very dark maroon or black. He calls them humin , formula $C_{40}H_{30}O_{15}$, and humic acid $C_{40}H_{24}O_{12}$.

In 1875, Tollens and Grotte (52) and in 1886, Conrad and Guthzeit (53) conducted research on the action of SO_4H_2 and HCl on various sugars and obtained humin , levulinic acid and the formation of the humic substances were simultaneous. They had treated the sugars during 15 to 20 hours, heating them with diluted acids and drying the humin at 130°. They verified then that certain sugars are much more sensitive than others to the action of acids.

From 1892 to 1905, Berthelot and André (54) confirmed and indicated in detail these results in a series of reports. They treated dextrose, levulose, galactose and maltose with more or less diluted acids HCl SO_4H_2 and PO_4H_3.

Their experiments were made:

a) in sealed, vacuum tubes, kept at 100°.

b) in oil-bath heated flasks:

1. condensing the water with a refrigerant which brings back the volatile products to liquid state;

2. with the aid of a descending refrigerant, restoring the water lost.

The experiments were made in vacuum, in air and under hydrogen atmosphere.

Determinations:

1. Unaltered glucose (imperfect Fehling determination because of the presence of glucosanes);
2. CO_2 (lime water);
3. CO (acid cuprous chloride);
4. humic acid (by washing, drying at 100° and weighing);
5. formic acid;
6. levulinic acid (method of division coefficients);
7. furfurole (through phenylhydrazine);
8. water.

In a second report, Berthelot verified that humic acids soluble in alkalis

are changed, by heating with 10% HCl, into humin insoluble in alkali, and then he studied the action of NH_4OH on humic acid, expressing it by the reaction:

$$3\ C_{18}H_{16}O_7\ +\ NH_3\ =\ C_{54}H_{45}O_{19}N\ +\ 2\ H_2O.$$
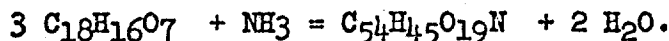
The presence of nitrogen in natural humin (1 to 2%) had already been indicated by Detmer (55) in 1871.

In 1912, Maillard (56) gave a very fruitful explanation in this connection and explained his theory in the course of various reports. He studied the reaction of glycocoll on the glucose -- 1 part of glycocoll, 4 parts of glucose, 3 to 4 parts of water to facilitate the solution. He heated in water-bath. The liquid becomes yellow after 10 minutes, and then its coloration increases until it becomes dark. CO_2 is released. This release results "from the splitting of the carboxyl and of the glycocoll; if we agree that this splitting is correlative of the fixation of N on the aldehydic carbon of the sugar, the molecules of the glucose, numbering 2 at least, which become a part of the new body, sustain dehydrations which create double liaisons and perhaps cycles".

The substances formed could be polycyclic molecules of an N atom.

He treated the glucose by means of glycocoll, sarcosine, alanine, valine, leucine, tyrosine and glutamic acid.

Alanine is the most active of the aminoacids.

Xylose and arabinose react instantly; fructose, galactose, glucose, mannose, quite rapidly; lactose and maltose, slowly.

Violent reaction at 150°, rapid at 100°, few days at 37°. This reaction is expressed by the following diagram:

$$CH_2OH\ (CHOH)_4\ CH: O + H_2N\ CH_2COOH\ =\ CH_2OH\ (CHOH)_4CH: N\ CH_2COOH + H_2O$$

$$CH_2OH\ (CHOH)_4\ CH: N\ CH_3 + CO_2$$

$$CH_2OH\ (CHOH)_4\ CH: O + H_2N\ CH_2\ COOH\ =\ CH_2OH\ (CHOH)_5\ NH\ CH_2\ COOH$$

$$CH_2OH\ (CHOH)_5\ NH\ CH_3 + CO_2$$
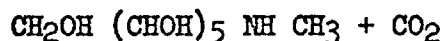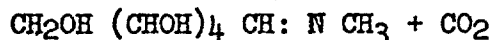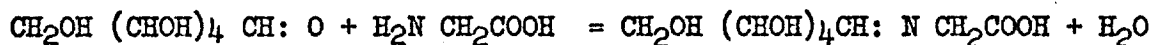
He noticed subsequently that the brown substances formed in these experiments "resembled those that sprout in the caramelization of sugars". The nitrogen content, 4.35% to 6%, was about the same as that of certain humic substances extracted from the ground and from melanoidic products; this is why he came to the conclusion that these substances were directly related.

He specified, besides, that the oxidation did not intercede in any way in the production of CO_2 and humic substances. He added that "the origin of mineral fuels could also be, to a certain extent, this reaction".

Finally, while studying "the formation of humic substances through the action of polypeptides on sugars", he noticed that "the polypeptide chain, regardless of its length, always terminates in two free ends NH_2 and COOH: it is desirable to know whether or not the intermediate groups prevent this molecule from reacting in the fashion of a simple aminated acid".

He took first a dipeptide, the glycyl-glycine:
$$H_2N\ --\ CH_2CO\ NH\ CH_2\ COOH$$
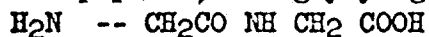
whose pure controlled samples originated from the partial hydrolysis of the cyclo-glycyl-glycine obtained through his method, and brought them together with xylose, the latter having been chosen because of its remarkable sensitiveness.

0.5 of glycyl-glycine, 2 grams of xylose and 3-4 cm^3 of water are placed in a small beaker maintained at 75°. At the end of 10 minutes, the yellow coloration is already quite visible; this coloration is strong after 10 minutes, almost brown after 20 minutes, and dark brown after 23 minutes. After 1 hour and 50 minutes, fine bubbles of CO_2 can be observed in the mass, and at the end of 2 hours this mass is copiously vacuolated. The phenomena go on as in the case of glycocoll: at the end of several hours, part of the product becomes insoluble and the entire product is transformed into a mass of insoluble skins if maintained in water bath for 2 or 3 days, replacing the small amount of water when necessary.

"The brown substance thus formed behaves exactly as that derived from glycocoll."

"The reaction also takes place at a lower temperature, but slower: at 40° it takes approximately 24 hours for the mixture to turn a dark brown color; about 34°, it takes 40 hours."

He got the same results with glucose and glycyl-glycine, but slower. The method of operation is quite similar. He added that the polypeptides were capable of interceding in the same manner as the amino-acids. Mallard had noticed previously that the dehydration reaction took place at a temperature considerably lower in the presence of amino-acids than in the course of the caramelization. This is verified when this is done in the presence of ammoniacal salts or ammonia, and in this connection we will mention at this time a report written by Ling and Nansit (57) even though it goes back to 1922.

The authors studied the action of NH_3 on the sugars in C_6 (glucose and levulose).

When glucose is treated through NH_3 at a weak temperature: approximately 35-40° and during a sufficient period of time, a combination of glucose and NH_3 is formed, which is called glucose ammonia. If this combination is then brought to a temperature of approximately 100°, an exothermic reaction is produced and blackish, strong-coloring substances appear. This glucose-ammonia combination is also produced by the action of amino-acids on the glucose, and it explains the coloration of the brown malts obtained by heating the malt at weak temperature, which malts are used in the manufacture of brown ale.

The glucose-ammonia reaction is the following:

$$
\begin{array}{lll}
CH(OH)_2 \cdots\cdots\cdots\cdots\cdots\cdots\cdots & CHOH --- & NH_2 \\
\quad | & \quad | & \\
CHOH & CHOH & \\
\quad | & \quad | & \\
CHOH & CHOH + & H_2O \\
\quad | \quad + NH_3 & \quad | & \\
CHOH & CHOH & \\
\quad | & \quad | & \\
CHOH & CHOH & \\
\quad | & \quad | & \\
CH_2OH & CH_2OH & \\
\text{Glucose} + H_2O \text{ (aldehyde hydrate)} & &
\end{array}
$$
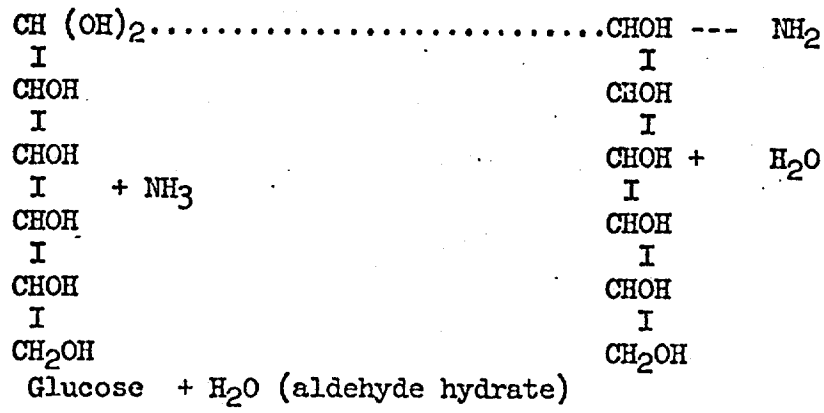
This reaction is said to be reversible; the appearance of the reaction depends on the glucose concentration of the solution used and on the NH_3 concentration; according to the author, a 20% would be needed.

The same reaction is said to be produced with levulose, but at a lower temperature, and, when heating at 62-76° brown, substances appear.

In 1915, Bottomley (58) studied the formation of humins in the hot treatment of glucose and levulose. By heating pure glucose during 4 hours at 180°, he obtained light brown-colored products perfectly soluble in water, or by adding strong acids he precipitated insoluble humins. If the temperature was raised to 220°, and the glucose was heated during 2 hours, the product obtained was no longer soluble, except in a diluted alkaline lye. The said product precipitated, however by adding HCl. When treating fructose, he verified that after heating for 6 hours at 120°, the dark brown-colored product obtained was soluble in water, but precipitated by HCl. After 2 hours of heating at 120°, the product was no longer soluble, except in alkalis. If the temperature was raised to 200° during 2 hours, the humin obtained was only insoluble in alkali. He noticed that the levulose was more sensitive to the raising of the temperature than the dextrose, which was already known.

In 1920, Fisher, Schrader and Treiss (59) transformed sugar humin, various types of carbon and cellulose by oxidation in alkaline medium, thanks to the oxygen in the air and to a high temperature.

For carbon and cellulose, they operated at a temperature of 250° and a pressure of 50 AE. For carbon, they obtained benzoic acid, isophthalic acid, carbo-benzoic acids and penta-carboxylic benzols. For cellulose, they obtained CO, CO_2, furanic acid and traces of oxalic acid.

With sugars, the preceding method had given them a red-brown, limpid solution in which the acids did not precipitate any body. They elevated the temperature to 400° and obtained then CO, CO_2, furan, aromatic acids, benzoic acids and phthalic derivatives. They concluded that the condensation of the aldehydic and cetonic groups into aromatic nuclei is effected in the same manner as mesetylene is obtained, starting from the acetone (Kane, 1838).

Eller (60) started, during the same period of time, a series of investigations in which he used concentrated NO_3H. His objective was to show the difference between phenol humins and sugar humins. By cold treating the hydroquinone humin through NO_3H, he obtained a substance soluble in alcohol, acetone and ethylic ether, but insoluble in the other organic solvents. This body was very hygroscopic. With water, the following reaction was obtained:

$$2 \ (C_6H_4O_3) \ X + (H \ NO_3) \ X = (C_{12}H_7O_6NO_2) \ X + (H_2O) \ X.$$
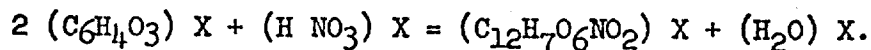
With hot diluted NO_3H, he obtained poorly defined products. With regard to NO_3H, the stability of sugar humin is greater than that of the phenol humin. The sugar humin dissolves at normal temperature in NO_3H, releasing rutilant fumes. After filtration, the unattacked fraction is dipped in water. It is soluble in alcohol and partially soluble in ether. It is a yellow, amorphous powder, and its immediate dissolution gives:

$$(C_{21}H_{21}O_9) \ X + (\ 2 \ HNO_3) \ \infty = (C_{21}H_{21}O_{14}N_2) \ X + (H_2O) \ X.$$
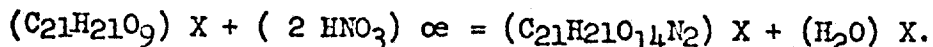
If the temperature is raised, the humin is decomposed. With hydroquinone humin, a rather large amount of oxalic acid is obtained. With sugar humin, traces of oxalic acid are formed.

In 1921, Marcusson (61), who was already known to the scientific world as an expert on humic products through a 1919 report, made a discovery which, although slightly distant from the theses disclosed by Midendorf (62) in 1917 with regard to the role of the oxymethilfurfurole in the composition of humic products, supported the idea of a furanic structure of the humin of any origin.

By treating furfurole through concentrated HCl, Marcusson obtained a black mass practically insoluble in anything. By melting it with KOH at 250°, he obtained CIK and humic acid. A demonstration was made, showing that furfurole with HCl condensated into humic substances. Williams Hoppeseyler and Koch (63) verified the release of furfurole in the treatment of cellulose. Furfurole is also obtained by treating bran through diluted SO_4H_2.

Beckley (64), in America, obtained in the same period of time similar results, but with oxymethylfurfurole.

In 1925, Burian (65) thought he could demonstrate, the presence of derivatives of furan and of furfurole in the distillates of natural or artificial humic acids obtained, respectively, by Cassel's brown and cellulose.

Marcusson thought that carbonhydrates arising from the degradation of vegetable residues would give furfurole. This compound gave benzoic groupings through a paradifurfuranic nucleus. The reaction is schematized as follows:
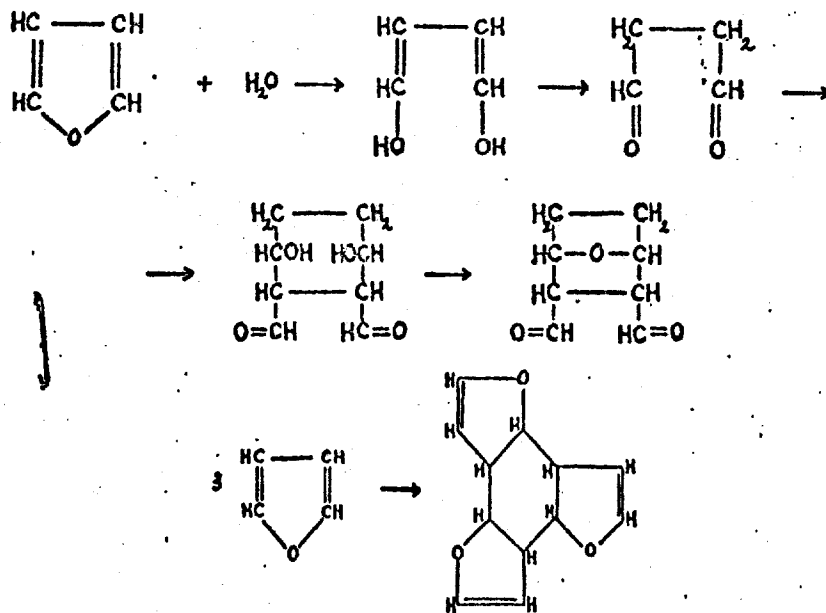
The black substance obtained by Marcusson (66) had been called caramelic acid, and it goes without saying that subsequent research was always influenced by a concern for showing the presence of this body. He used particularly hydrogen peroxide with traces of SO_4Fe in order to obtain it starting from Merck's humic acid.

Francis and Wheeler (67) studied the action of hydrogen peroxide on carbon in alkaline solution.

They obtained products soluble in alcohol, acetone and pyridine. Bone, Horton and Ward (68), during the same period, oxidized the carbon through potassium permanganate and obtained humic acid, oxalic acid, CO_2 and polyacid benzol.

In 1926, Fuchs (69) and his collaborators started a series of experiments of great importance. They used the nitrous products in order to bring to light the constitution of the humic products. They agreed that the nitrogenous liasons were directly related to the humin itself.

They called "dehydrohumic acid" the body obtained in their research, and classified the natural hymatomelanic acids in this category. They established the molecular weight of this substance thanks to its solubility in acetone.

By proceeding to the nitration, they found an increase of 2 to 3% in the content of N. This figure is close to the N content of natural humic acid, which is 3 to 5%.

Fuchs thought then that isonitrosylic groups were formed. After several boilings in the presence of HNO_3, Fuchs and Stengel (70) obtained, with a little oxalic acid, several benzenic nucleus acids, with 4 to 5 carboxyl groups, picric acid and other nitrophenols. Fuchs tried to methylate the nitrous product completely by heating it first with HCl and methylic alcohol and then by setting diazomethane to work. He tested the groups present and obtained the formula:

$$C_{59}H_{36}O_{17}N \, (COOCH_3) \, 4 \, (OCH_3) \, 3 \, (C = NOH) \, (CO).$$
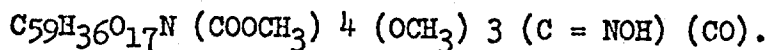
He concluded that the following groups were present in the natural humic acids:

    4 COOH groups;
    4 OH groups;
    1 CH_2CO;
    2 O groups;
    1 CH: CH group;
    1 group H replaceable
    4 to 8 groups H dehydratable

Fuchs (71) prepared methylated oxide ethers of alcoholic functions of sugar humins, and compared the various humic acids with regard to the absorbed NH_3 and the relations starting from NH_4 changeable to NH_3.

These comparisons were made between Merck's humic acid, sugar humic acid and hydroquinone humic acid.

Natural humins and sugar humins are closer to each other with regard to their properties than natural humins and polyphenol humins.

Besides the oxidating action, the halogen action, that of the halogen groupings was used in order to bring to light the similarity of the natural, sugar and phenol humins. Fuchs (72) and Eller (73) studied the action of Cl and Br. Schmidt and Atterer (74) studied the action of ClO_2 with a vanadium salt as catalyzer, on humic products in alkaline solution. They obtained maleic acid attributed to a group - C - CH: CH C or - C: CH - CH: C - .

These combined C_4 are found in the $C_6 H_6$ nuclei or furanic nuclei.

Furfurole treated through cold concentrated HCl gave almost immediately a release of humin which could be exposed with ClO_2.

In 1927, Schmidt and Atterer (74) thought that there was an enormous difference of structure between the furan humins and the other category humins, and they wondered if the sugar humins could be regarded as furan derivatives, since the changing of the furfurole into humin could be either the result of a simple polymerization

or the result of a noticeable change.

The matter was taken up again later by Page and Du Toit (75) in 1932.

Berl and Schmidt (76) studied the formation of carbon beginning from cellulose and glucose by heating with water and under pressure at 225° and 250o. At 250°, they obtained 27% humin and 73% carbon.

Willstatter and Kalb (77) obtained identical results in reducing medium at 250° with glucose humin, cellulose, glucose and xylose.

Orlow and Tichenko (78) studied sugar carbons by proceeding at a pressure of 170 AE and at a temperature of 300°, and then at a pressure of 100 AE and a temperature of 400/440°. Then they proceeded to the NO_3H action on this product.

In a parallel direction with this research, the more and more precise establishing of the physical characteristics of the products obtained, and particularly the elementary weights b osmotic pressure -- Samec (79), C. Arnold (80) -- diffusion -- Zeile (841), Enders (82), Schelle (83) -- cryoscopy in acetone -- Fuchs (84), Eller (85), Stach (86), Kurschner (87) -- basicity, constituted one of the strongest reinforcements of the study of humic substances, indeed of analytical tests.

This account has led us to a date that is extremely close to that closing chapter 1. This is due to the fact that, since 1900, the caramel research devoted more and more time to humic substances. The methods of detection used were directly inspired by the reports of experts on soil or carbon chemistry (action of diluted or concentrated acids, oxidants, halogens, alkalis, etc.).

Having succeeded, thanks to the agreement of the results and the establishing of standard methods, in showing and controlling the mechanism of the formation of humic substances beginning from carbon hydrates, it was understandable that the isolated study of the caramel and the humic products derived from the sugar was practically completed, and that a good scientific explanation could not be found except within the frame of hypotheses and research relative to the chemistry of humus. This is what leads us to the third part of our account.

CHAPTER III

Recent theories that adhere more specifically to the present two commercial classes of caramel.

We have brought our two previous summaries to approximately the year 1936. At that time, Selman, A., Wacksmann (88) fixed the theories as three, "attempting to explain the formation of black substances in the humus".

The humus was said to be due:

1. to the reaction of the acids on the carbon hydrates.
   Chemical conception based on Marcusson's, Beckley's and Burian's theories.
   The sugars in C_5 give furfurole, and the sugars in C_6 give oxymethyl-furfurole.
   This theory was not supported by Eller and Schmidt;
2. to the condensation of the carbon hydrates by the aminated acids or polypeptides.
   Maillard's theory;
3. to the oxidation of benzoic nucleus compounds. Hoppe-Seyler's Reinitzer's (89) and Eller's theory.

Since that time, a considerable amount of research work has been done, and we will borrow data only from the remarkable book published by Fritz Scheffer and Bernhard Ulrich (90), which contains a summary of the latest theories on this subject. These theories are explained in two chapters which we will reproduce to a large extent.

The authors point out that the preparation of the humic substances beginning from the sugars or cellulose has been effected either through the action of strong mineral acids, concentrated under determined conditions or through heating (caramelization), Stadnikow (91), Bergstrom (92), Thiele (93).

Sugars and uronic acids (aldehyde acids of general formula: HO_2C -- $(CHOH)_4$ CHO, derived from the aldoses in C_6) formed in the course of reaction, give among others:

1. reductones;
2. α-oxycarboxyl groups;
3. furanic derivatives.

The reductones and the α-oxycarboxyl groups are formed particularly in neutral alkaline solution or slightly acid solutions. The reductones have acid characteristics (Enders (94)).

REDUCTONES - They have the following atomic group:

$$-C=\begin{bmatrix}H & H\\ C-C\end{bmatrix}_n=C-C=*$$
$$\overset{OH}{|} \qquad \overset{OH}{|}$$

LXIX (n = 0 4)
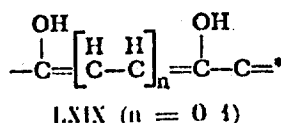
According to Euler (95), "the first step of the oxidation of the Endiol group (LXX) would be the release of 2 electrons, thus giving rise to the biradical LXXI which, through displacement of the electrons, is changed into a dicetonic LXXII group of dehydroreductones the dicetones being of a strong yellow coloring. This formula bears a resemblance to the phenoquinone reactions".

The reductones are of a great biological importance. The "Endiol" group exists in substances favoring growth and its inhibitors. Vitamins (ascorbic acid), hormones (adrenalin).
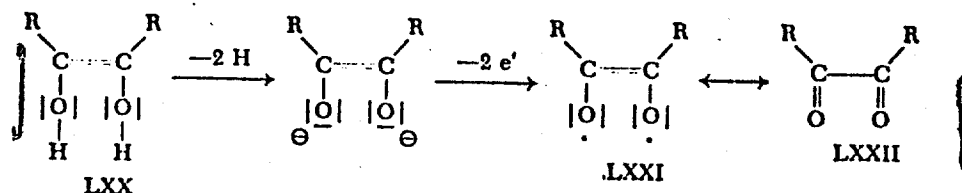
The true or potential α-oxycarboxyl groups representing the products of double decomposition of sugars are:

CH_2OH -- CHO (glucolic aldehyde)
CH_2OH -- CHOH -- CHO (glyceric aldehyde)
CH_2OH -- CO -- CH_2OH (dioxyacetone)
CH_3 -- CO -- CO -- CH_3 (diacetyldicetone).
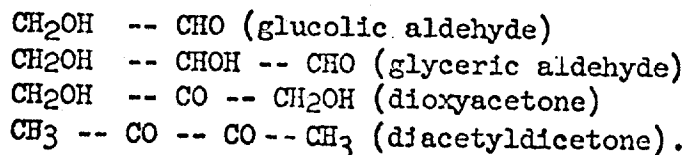
These products are capable of forming humic substances in aqueous solutions.

Enders (96) proved that the reducing triose CHOH = COH --CHO was capable of cyclic synthesis, thus demonstrating the development of the quinonic cycles. Besides, he assigned a principal role to an oxido-reducing triose with respect to the glyceric aldehyde, the dioxyacetone and the glyoxal methyl.

Flaig (97) proposed the following diagram:

Beginning from the methylglyoxal or propanalone (aldehyde cetone), CH_3 -- CO-- CHO (pyruvic aldehyde):

LXXVII   .I
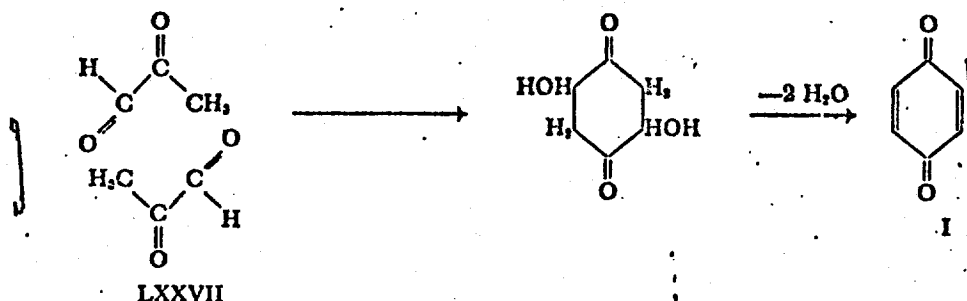
FURANIC DERIVATIVES (Fisher (98), Marcusson (99), Eller (100)).-Polymerization of sugars occurs already in strongly diluted or weakly acid solution. Furfurole - forms a peroxide with the oxygen in the air. All the furanic derivatives are auto-oxidizable under the influence of the oxygen in the air and give brown products which are responsible for the coloration of molasses.

These three paragraphs deplete, it seems, our present knowledge regarding caramelization proper or pyrogenation of sugars.

The appearing, at the beginning of the century, of coloring products derived from sugar, under conditions practically defined by Salomon (A.G.) and Coldie (E.L.) (101), leads us to explain recent theories concerning Maillard's reaction, to which the bodies obtained under these conditions adhere.

Scheffer and Ulrich (102) point out that these products precipitatable in acid medium are melanoidins. The aminated groups catalyze the double decomposition of the sugars (depending on whether the products formed are regarded as due to an al-dolyzation or to a de-aldolyzation) under the form of aldo-condensation or de-aldo-condensation. Nitrogen can also be included in these reactional products. The role of the aminated groups is reduced to a catalytic effect. These conditions resemble more or less those of the aminated groups which react on the phenols.

The double decomposition of sugar catalyzed through aminated groups starts with the addition of RNH_2 to the existing aldose (LXXXVI). The addition body LXXXVII is changed into a Schiff's basis by losing the H_2O (LXXXVIII). The latter is changed into glycosylamine with an N replaced IXC.

By passing through the cation of Schiff's base (cation XC), the following is formed: 1-amino-1-deoxy-2-cetose with N replaced in the group ENOL XIC, possibly in the group Ceto XIIC (relating to the (Amadori) transformation), which is, as we know, "the conversion of the N glucosides of aldose into aminated derivatives of the corresponding cetones" (103).

In the configuration 1, 2 ENOL, the liaison C -- C into α or β position is weakened h the carboxyl group. This is why the aminated groups catalyze the aldose reduction into trioses and other products of double decomposition.

Beginning with the 1-amino-1-deoxy-2-cetose XHC, we arrive, in a neutral or acid aqueous medium through double decomposition of H_2O (catalyzed by the acids), at the formation of Schiff's bases derived from furfurole LXXX or from oxymethyl-furfurole LXXXII, from which the aminated groups can be separated by fixing water.

In systems without water, on the other hand, reductones are preferably produced by double decomposition of water.

We find again in both cases products of double decomposition of sugars, of whith we have already spoken (flucolic aldehyde, glyceric aldehyde, etc.).
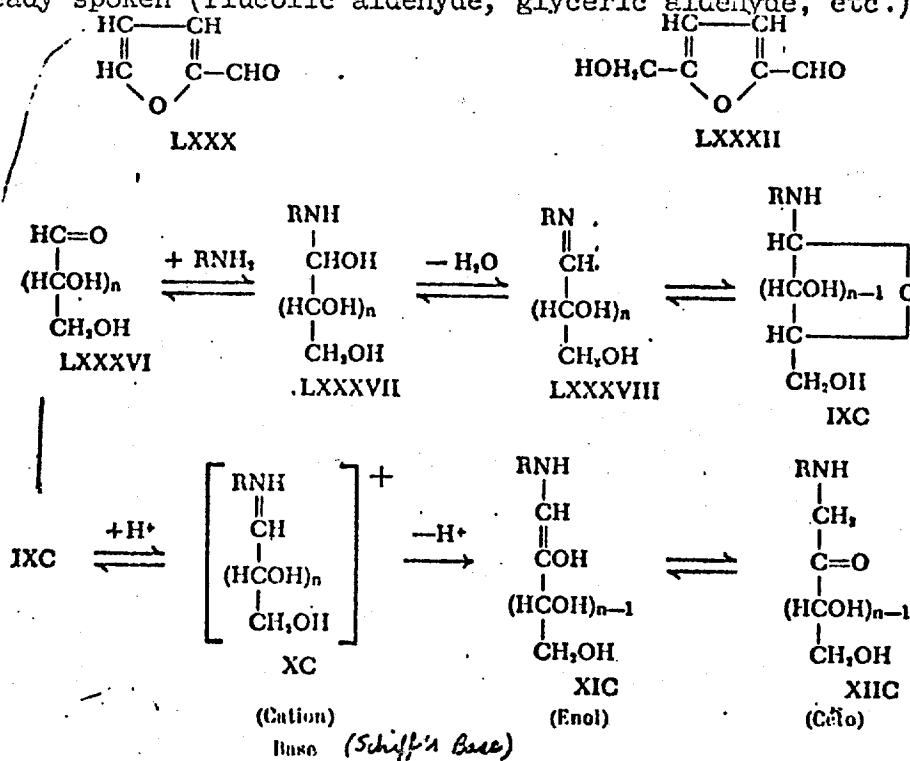
Concomitantly, and in large proportion, the amino-acids are changed into corresponding aldehydes through reaction on the groups of dicetonic liaisons with release of CO_2 (Strecker's degradation) (104).

This is the reason for the following diagram:
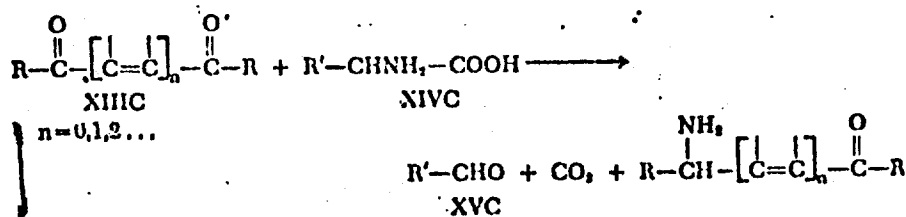
In all cases, there is a more or less important osidic fraction. In the case where the thermic action on the sugars is conducted with a sparing addition of concentrated alkalis such as KOH, NaOH, etc., the end reaction is practically derived from the formation of the reductone triose (105):
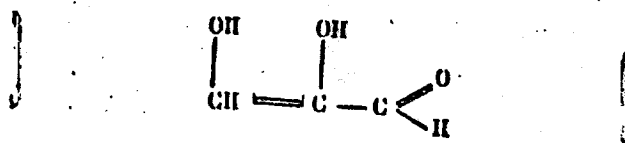
which, in aqueous medium, gives a balanced system with its tautomer of equal weight, the hydroxymalonyldialdehyde:
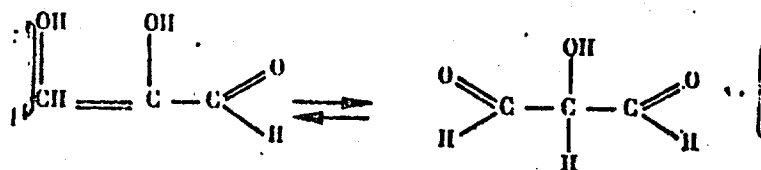
this is why the quinonic cycles predominate.

In the case where the thermic action takes place in the presence of diluted mineral acids (HCl, SO_4H_2, etc.) or organic acids (citric acid, tartaric acid, etc.), we have a formation (106):

beginning from the furfurole pentoses;

beginning from the hydroxymethylfurfurole hexoses:

Pentose   Furfurole

CH₃.CO.CH₂.CH₂COOH acide lévulique (*levulic acid*)
+
H.COOH   acide formique (*formic acid*)
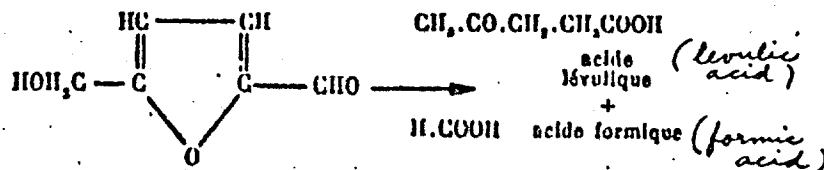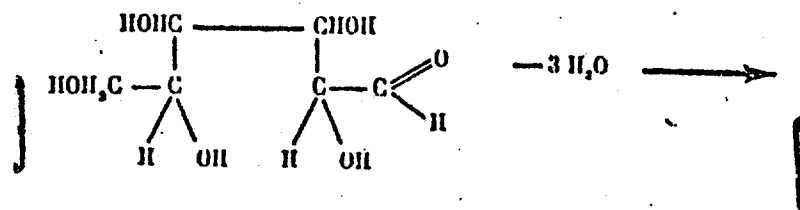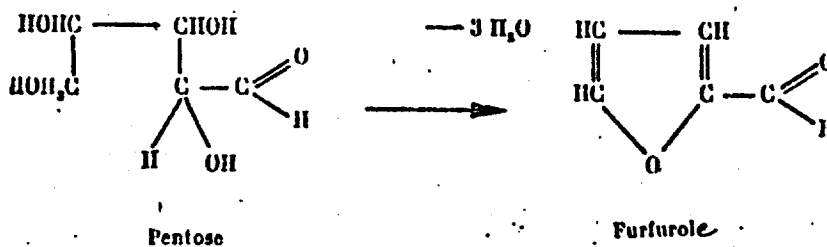
The thermal action conducted in a sparing manner and in autoclave, in the presence of NH_3 or ammoniacal salts, gives rise to the formation of bodies derived from Maillard's reaction, schematized by Hodge (107) as follows:
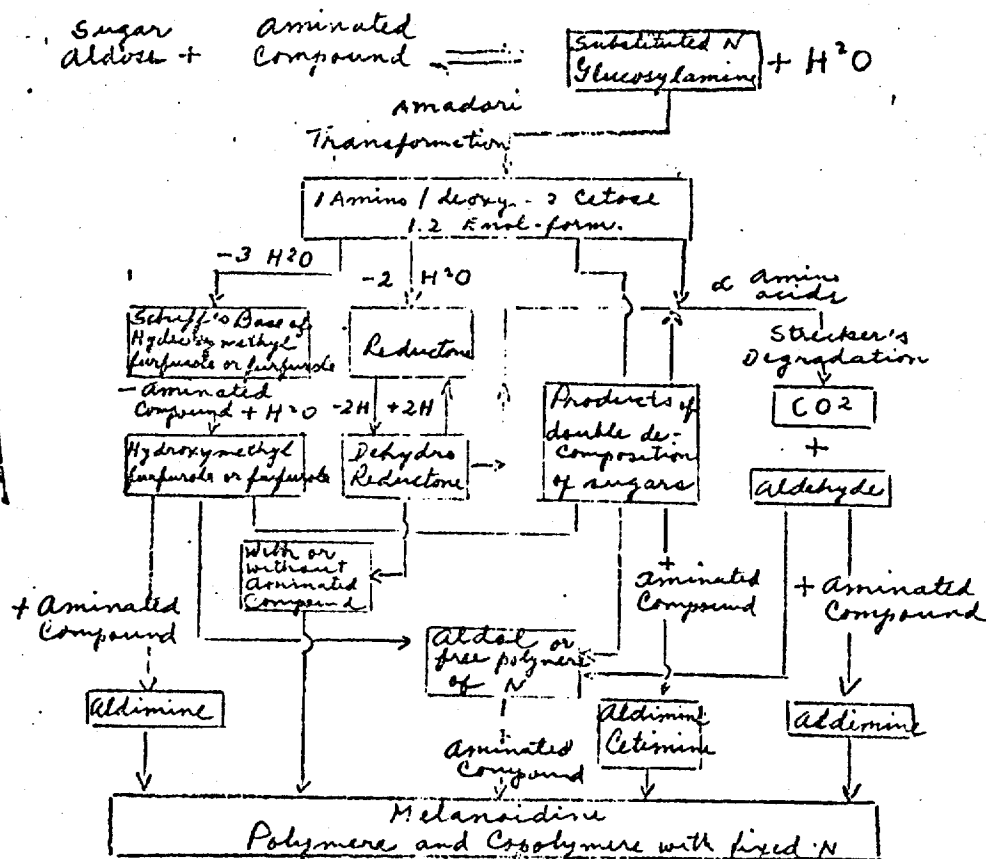
The browning of food substances being due to four main reactions (108):

1. Enzymatic or non-enzymatic oxidation of the polyphenols;

2. Thermal transformation of the proteins and their basic elements;

3. Pyrogenation of carbon hydrates;

4. Maillard's reaction,

it is verified that the origin of the formation of products composing the caramels is to be connected with the latter two reactions.

The elements exposed above provide, therefore, a valuable foundation for the interpretation of reactional phenomena which assure the obtaining of coloring substances beginning from the sugars.

The formation of iso and heterocyclic nuclei is effected beginning with the reductones in the presence of amines and aldehydes as per the following diagram:

Leading to pentagonal and hexagonal nuclei.

In summary, the polymerization of sugars through thermal action alone gives rise to the formation:

1. of reductones;
2. of α-oxycarboxyl group;
3. of furanic derivatives: quinone, furfurole, oxymethylfurfurole.

This effected in the presence of catalyzers, such as the aminated acids, gives:

1. reductones;
2. aldehydes (arisen from the degradation of the α-amino-acids);
3. pentagonal and hexagonal cyclic bodies: quinone, furfurole oxymethylfurfurole and imino derivatives and amino-Pyrrol, Imidazole, Pyridine, Pyrazine.

The results mentioned are, however, those obtained from scientific research far removed from the industrial procedures used for the quantities or the quality of the products created as well as for the operating conditions.

The industrial methods too, have been the subject of research and publications which we intend to cover in a second section that will also comprise the main physical as well as chemical detection and characterization reactions of the caramels.

The mentioning of patents registered to this date regarding the material or the manufacture of coloring substances derived from sugars will complete this second study.

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Caramel

ÉTUDE BIBLIOGRAPHIQUE DU CARAMEL (*)

Par MM. R. TRUHAUT, G. VITTE et V. LASSALLE-SAINT-JEAN.

Les auteurs rapportent les différentes réactions chimiques pratiques et théoriques donnant lieu à la formation de colorants noirs du type « Caramel ». Deux réactions fondamentales du brunissement des matières alimentaires ont retenu leur attention : la pyrogénisation des hydrates de carbone et la réaction de Maillard. Ils exposent dans chaque cas la dynamique chimique et la nature des produits de polymérisation obtenus soit en général, soit dans des cas particuliers.

Les historiens s'accordent à fixer à l'époque des Croisades (1230 environ) l'introduction de la canne à sucre en Europe et sa culture en Sicile.

Les produits extraits de la canne à sucre étaient connus dans le Bengale et le Moyen-Orient depuis la plus haute antiquité.

Les Romains en donnaient à leurs athlètes. Néanmoins, ce n'est que vers le VIᵉ siècle après Jésus-Christ que s'instaura un véritable commerce dans ce domaine, apanage exclusif durant longtemps des Républiques italiennes.

Les caravanes arabes transportaient cette denrée. Sa valeur était liée à sa coloration. Les marchandises les plus colorées étaient les moins appréciées. Cette dernière qualité avait le nom de « curat milh » signifiant, en arabe : ballot sucré. On suppose que c'est à cela que l'on doit le nom de caramel qui a servi à désigner le produit brun noir issu de la pyrogénisation des sucres.

Dans l'industrie sucrière, le caramel existe dans une proportion plus ou moins grande dans toutes les fabrications; il se trouve, selon les techniques de préparation, à la dose de 0,4 à 0,5 % [1] dans le sucre. Comme sa présence constitue une perte, le dépistage et le contrôle de sa formation font l'objet de recherches de la part de techniciens spécialisés.

Cependant si, en ce qui concerne l'industrie sucrière, la présence d'une forte proportion de caramel ne se rencontre qu'à des occasions exceptionnelles, dans l'industrie du glucose, par contre, comme celui-ci s'obtient généralement en faisant agir un acide fort dilué sur des fécules, les risques de caramélisation constituent une préoccupation constante.

Le fructose étant plus particulièrement sensible à la réaction thermique, le caramel constitue également, dans ce cas, la cause de perte et de pollution particulièrement surveillées.

Cependant, avant toute chose, le caramel est un colorant. Sa définition est donnée comme matière colorante d'origine naturelle dans l'annexe I de l'arrêté officiel du 18 juin 1958 : « Colorant obtenu par chauffage du sucre à une température supérieure à son point de fusion, mais sans pousser la caramélisation jusqu'à une masse compacte brune ou noire. »

Son goût est généralement amer, cette amertume étant augmentée par addition de petites quantités d'alcali ou de carbonates alcalins introduits en cours de fabrication.

Il se présente comme un liquide visqueux dont la couleur varie du brun foncé au noir, possédant une odeur caractéristique.

Le Codex pharmaceutique américain [2] exige, pour le caramel, les obligations suivantes :

— densité égale ou supérieure à 1.3 prise à 25° C.;
— il est soluble et sa solution aqueuse à 1 % doit avoir une teinte sépia clair qui doit se maintenir un minimum de six heures et ne pas précipiter pendant le même temps d'exposition à la lumière solaire. Ce caramel est soluble à toute proportion dans l'eau;
— il est également soluble dans l'alcool de moins de 55°, mais insoluble dans la plupart des solvants organiques, tels que l'éther éthylique, le pétrole, la benzine, le chloroforme, l'acétone, etc.

Comme il sera précisé plus loin, le caramel est un mélange de corps. Préparé à partir du saccharose, il est complètement soluble dans l'eau. Néanmoins, si le sucre n'est pas raffiné, des composés insolubles apparaissent. La quantité de ces composés croît avec la déshydratation.

Ce caramel est plus soluble dans les alcools que celui préparé à partir des glucoses, par suite de la présence de dextrine dans ces derniers.

L'addition de carbonates alcalins durant la cuisson augmente le pouvoir colorant et favorise la solubilité de certaines fractions qui seraient insolubles sans cette présence. On utilise également des caramels solides ou pulvérisés. Le caramel solide est généralement brun rouge, cassant, amorphe et très déliquescent.

COMPOSITION CHIMIQUE DU CARAMEL

Les concepts scientifiques et les études afférentes à la composition chimique du caramel ont subi depuis le début des recherches jusqu'à nos jours une évolution extrêmement sensible.

Un exposé chronologique unique risquant d'entraîner une confusion regrettable ou fastidieuse susceptible de dérouter tout lecteur non averti, nous adopterons une présentation scindée en trois parties.

Un premier chapitre traitera des recherches ayant le caramel pour origine et raison.

Un second procédera plus particulièrement du comportement des sucres sous l'influence de certains facteurs physiques ou chimiques.

Un troisième mentionnera les théories récentes s'attachant plus spécifiquement aux deux catégories commerciales actuelles des caramels.

CHAPITRE PREMIER

Recherches ayant le caramel pour origine et raison.

Dès 1838, Eugène Péligot [3], dans un article sur les *Recherches sur la nature et les propriétés chimiques des sucres*, étudie « l'action de la chaleur sur les sucres ». Il note l'existence d'une substance en mélange avec le sucre qu'il désigne sous le nom de caramel « afin d'éviter la création toujours embarrassante d'un nom nouveau » et à qui il attribue la formule : $C_{12}H_{10}O_{11}$.

Il signale la présence d'acide acétique dans l'eau de condensation issue de la pyrogénisation des sucres, précise le caractère acide du produit, mentionne sa précipitation par l'acétate de Pb ammoniacal et l'eau de baryte.

Le caramel ne sera pratiquement plus mentionné dans la presse scientifique jusqu'en mars 1858, époque à laquelle A. Gélis [4] publie son *Etude du caramel et des produits torréfiés*.

Il signale que Voelkel [5] avait répété et confirmé les expériences de Péligot et donné le nom de Caramélan au produit colorant en lui attribuant la formule : $C_2H_{13}O_{13}$.

Mais ses travaux avaient pour origine « la recherche de « l'assamare » (de *assare* : griller, rôtir, et de *amarus* : amer) dans les goudrons qui distillent lorsqu'on décompose complètement le sucre par le feu ». Ce terme d'assamare était dû à Reichenbach [6] et désignait un principe amer prenant naissance toutes les fois que les matières organiques sont chauffées jusqu'à ce qu'elles brunissent.

A. Gélis voulut « approfondir la question » d'autant plus que Gerhardt [7] avait supposé que l'assamare de Voelkel était identique à « l'acide apoglucique » de Mulder [8]. Cette besogne nous valut une étude concluant à l'existence de trois substances fondamentales dans le caramel : la Caramélane, la Caramélène, la Caraméline.

1° LA CARAMÉLANE. — Pour obtenir ce corps, Gélis traite le caramel issu de la cuisson du saccharose à une température de 210 à 220° par de l'alcool à 84°. Il conserve la fraction alcoolique, évapore l'alcool à une température inférieure à 120°, reprend par l'eau et laisse fermenter la fraction des oses existant, en présence de levure de bière.

Après concentration, la Caramélane est précipitée par l'alcool absolu. L'établissement de sa formule est fait par le traitement à l'acétate de plomb et les sels de plomb répondent à la formule : $C_{12}H_{16}O,PbO$. Il a également obtenu une combinaison avec le baryum.

2° LA CARAMÉLÈNE. — « Le caramel ordinaire, épuisé par l'alcool à 84°, est entièrement privé de la Caramélane et laisse un résidu qui, traité par l'eau distillée froide, se dissout en partie; « la matière dissoute est de la Caramélane presque pure ».

Dans ce cas-là également, il est fait usage de combinaison de plomb ou de baryum, grâce à laquelle on parvient à une formule $C_{36}H_{48}O_{24}H_2O$.

A noter l'existence de trois combinaisons plombiques :

une première, obtenue en milieu acide acétique correspondant
à la formule .... $C_{36}H_{48}O_{24}$ PbO
une deuxième, en milieu ammoniacal fort .... $C_{36}H_{48}O_{24}$ 5 PbO
une troisième, en milieu ammoniacal faible .... $C_{36}H_{48}O_{24}$ 6 PbO

3° LA CARAMÉLINE. — Après les deux traitements précités, il reste un résidu. « Il ne contient qu'un seul corps, la Caraméline, mêlée à des quantités variables de sucre plus ou moins charbonné, mais cette Caraméline existe sous différents états isomériques. »

Elle existe sous trois formes :

— une première partie soluble dans l'eau froide;
— une deuxième soluble dans l'eau chaude et l'alcool à 60 %;
— une troisième insoluble, mais partiellement soluble dans les alcalis.

L'ensemble de ces produits donne toujours un précipité, tant avec $BaCl_2$ qu'avec l'acétate de Pb, et Gélis considère que la formation de ces divers

produits est directement liée à la perte de poids en eau du saccharose. Il établit l'échelle suivante :

Pour une réduction de :

10 % on obtient de la caramélane $= C_{12}H_{22}O_{11} = C_{12}H_{18}O_{9} + 2 H_2O$;

11 % on obtient de la Caramélène $= C_{36}H_{66}O_{33} = C_{36}H_{50}O_{25} + 8 H_2O$;

25 % on obtient de la Caraméline $= C_{12}H_{22}O_{11} = C_{12}H_{12}O_{6} + 5 H_2O$.

En outre, Gélis, ayant traité à chaud tous ces produits, issus soit de caramel de saccharose ou de glucose, par de l'acide azotique, a obtenu de l'acide oxalique.

En 1862, Thomas Graham [9] publiait des travaux sur *La diffusion moléculaire appliquée à l'analyse.*

Etudiant le caramel brut obtenu en chauffant du sucre de canne à 210/220°, il constatait que l'examen dialytique de cette matière venait corroborer les notes communiquées par A. Gélis.

La Caramélane et la Caramélène de Gélis diffusent et la substance qui reste sur le diaphragme présente un pouvoir colorant égal à 5 fois le caramel brut primitif.

On peut obtenir ce corps en le précipitant par l'alcool de ses solutions aqueuses.

Desséché à basse température, ce caramel soluble peut être ensuite chauffé à 120° sans cesser d'être soluble, mais si une solution de ce même caramel est évaporée à siccité, directement, sur le bain-marie, le résidu obtenu est entièrement insoluble dans l'eau froide ou chaude. Soluble ou insoluble, ce corps a même composition.

Graham note le caractère colloïdal du produit. Il propose la formule : $C_{24}H_{15}O_{13}$, et il ajoute :

« L'analyse donnée par Gélis de sa Caraméline ne s'applique en rien au composé dont nous nous occupons. » [10].

Il constate que ce caramel a une sensibilité excessive à l'action des réactifs cristalloïdes. Il est précipité ou coagulé par de simples traces d'acide minéral, les sulfates alcalins, par ClNa, ainsi que par l'alcool.

Fait important, la présence de sucre ou de produits peu déshydratés neutralise l'action des cristalloïdes.

Cette constatation sera reprise par d'autres auteurs dans leurs travaux.

Ce caramel est précipité néanmoins par certaines substances de sa propre famille, comme le peroxyde de fer.

Il note que la lenteur de diffusion doit être rapportée à la substance qu'il vient de décrire.

Les autres produits qui l'accompagnent, plus diffusibles, le sont cependant moins que toutes les variétés de sucre.

Le caramel est 600 fois moins diffusible que ClNa et 200 fois moins que le sucre.

Graham signale l'analogie que le caramel, sous sa forme insoluble, présente avec la houille; la caramélisation pourrait être le début d'un processus colloïdal devant se compléter dans la longue période des époques géologiques pour parvenir au charbon.

En 1899, Stolle [11] étudiait à son tour la pyrogénisation des sucres, et dans une suite d'articles publiés de 1899 à 1903, arrivait aux conclusions suivantes :

Le produit principal est la Caramélane de Gélis, obtenue par perte de 12 % du poids initial dans le chauffage du sucre, celui-ci étant porté entre

180 et 190°. Jusqu'à 190°, le processus s'effectue uniquement par perte d'eau; au-dessus, il y a dégagement de CO_2 et d'acétone.

La combinaison monoplombique ne précipite ni en milieu neutre, ni acétique faible, mais uniquement en milieu alcalin, ce qui ne correspondait pas aux conclusions de Gélis.

Il en a tiré la conclusion que lors de l'analyse de la mélasse la clarification étant obtenue par l'acétate de plomb, la Caramélane non précipitée pouvait réduire la liqueur de Fehling; ladite réduction imputée faussement au sucre constituait une cause d'erreur.

Il a proposé la formule $C_{12}H_{18}O_9$ pour ce produit avec lequel il a préparé un tétracétate et un monobenzoate.

La Caramélane, hydrolisée en la faisant bouillir 18 heures avec SO_4H_2 dilué, voit sa solution foncée devenir jaune clair avec formation de flocons d'acide humique pour lequel Stolle propose la composition : $(C_8H_4O_3)^x$, ainsi que de l'acide lévulique et une hexose semblable au dextrose, mais qu'il n'a pas identifiée.

En 1906, Trillat [12], étudiant les produits volatils formés lorsque le sucre est chauffé, constatait qu'à :

200° 0,1 à 0,3 % de formaldéhyde était obtenu;

100° des traces sont apparentes après 24 heures;

150° on la détecte dans quelques minutes.

D'où sa conclusion que le caramel était réellement une combinaison de produits polymérisés de formaldéhyde.

En 1909, Ehrlich [13], au cours de recherches, réalisa la caramélisation du sucre « dans le vide ». Il estima que la matière colorante du caramel pur sucre est un composé $C_{12}H_{22}O_{11} - 2H_2O$ qu'il appelle « saccharone ».

Il obtient ce produit par cuite du sucre dans un flacon immergé dans l'huile à 200° C. Le résidu restant après extraction des autres composés par l'alcool méthylique fut dissous dans l'eau, filtré et évaporé jusqu'à obtention d'un corps amorphe brun noir et facilement réduit en poudre.

Ces travaux servirent de bases à d'autres chercheurs, la méthode de caramélisation du sucre « sous vide » offrant des garanties scientifiques certaines.

En 1917, Mary Cunningham et Charles Dorée [14] publiaient une *Contribution à la chimie du caramel*, inspirée par les travaux de Gélis et de Stolle.

Dès le début de leur article, s'appuyant sur les travaux de Green [15], Willstätter [16], Fenton [17], Fisher [18], Sestini [19], Bottomley [20], ils proposent, à la place des formules établies par Gélis pour la Caramélane, la Caramélène et la Caraméline (à qui on peut reprocher une teneur en eau arbitraire), les formules suivantes comme étant les seules valables :

Caramélane : $6 C_{12}H_{22}O_{11} - 12 H_2O = 6 C_{12}H_{18}O_9$;

Caramélène : $6 C_{12}H_{22}O_{11} - 18 H_2O = 2 C_{36}H_{48}O_{24}$;

Caraméline : $6 C_{12}H_{22}O_{11} - 27 H_2O = 3 C_{24}H_{26}O_{13}$.
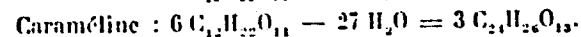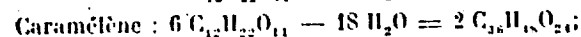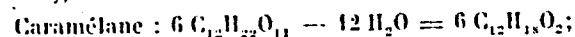
Suivant le procédé de Stolle, ils chauffèrent le sucre entre 180° et 190° :

La Caramélane fut obtenue avec une perte de poids de 12 %;

La Caramélène fut obtenue avec une perte de poids de 15 %;

La Caraméline fut obtenue avec une perte de poids de 22 %.

La Caramélane obtenue par ce traitement est un solide brun, cassant, amer, sans odeur, très hygroscopique, fondant à 136° et s'amollissant à 100°, soluble

dans l'alcool à 84°, la pyridine, l'alcool méthylique, l'acide acétique cristal-lisé chaud et insoluble dans l'éther, l'alcool pur ou le benzène. Elle réduit la liqueur de Fehling et le nitrate d'argent.

Les solutions aqueuses acidifiées avec HCl donnent un précipité lors de l'addition de résorcine, qui est soluble dans l'alcool et les alcalis. La phloro-glucine donne un précipité semblable, mais de coloration plus intense.

Ils ont procédé par la suite à une série d'études.

1° LES ESTERS DE LA CARAMÉLANE. — Ils ont obtenu :

  — un tétraacétate;
  — un tétrabenzoate;
  — un tétranitrate;

— un tétraacétate jaune ayant pour point de fusion 107°, insoluble dans l'eau ou l'éther, soluble dans C_6H_6, l'alcool chaud ou l'acide acétique glacial, réduisant le Fehling;

— un tétrabenzoate coloré en chamois pâle ayant pour point de fusion 105 à 108° soluble dans l'acétone, $CHCl_3$, l'alcool, C_6H_6, insoluble dans l'eau, l'éther et le pétrole léger;

— un tétranitrate jaune, qui s'enflamme violemment sous l'influence de la chaleur, qui est rapidement soluble dans l'éther ou le benzène, inso-luble dans l'eau.

Ils ont déterminé les poids moléculaires de la Caramélane et de ses esters.

2° ILS ONT ÉTUDIÉ LE CARACTÈRE ALDÉHYDIQUE OU CÉTONIQUE DE LA CARAMÉLANE.

A. Avec la Phénylhydrazine :

a) en présence d'acide acétique :
$$2 C_{12}H_{14}O_9 + C_6H_5 NH NH_2 = C_{30}H_{34}O_{15}N_2 + 3 H_2O;$$

b) en présence de SO_4H_2O :
$$2 C_{12}H_{14}O_9 + C_6H_5 NH NH_2 = C_{30}H_{26}O_{16}N_2 + 8 H_2O.$$
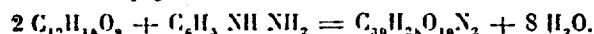
B. Avec la semi-carbazide :
$$3 C_{12}H_{14}O_9 + NH_2 NH CONH_2 = C_{37}H_{43}O_{20}N_3 + 8 H_2O.$$
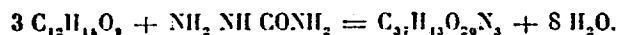
3° ACTION DES ACIDES SUR LA CARAMÉLANE. — S'appuyant sur les travaux de Conrad et Guthzeit [21], Bottomley [22], Stolle [23], Krober [24], Eller et Tollens [25], Cross [26], Gostling [27], Sestini [28], Willstatter [29] et Fisher [30], ils établirent une série de résultats consignés dans le tableau ci-dessous :
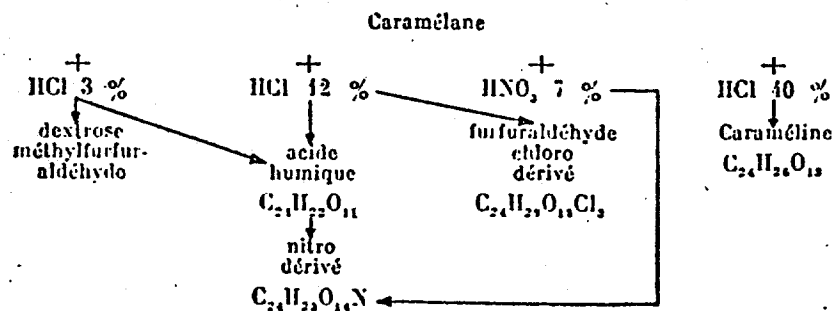
Caramélane

4° ACÉTOLYSATION DE LA CARAMÉLANE. — Rappelant les travaux de Skraup [31], Born et Nelson [32], les auteurs concluent que la Caramélane est soit un mono ou un dissacharide.

5° L'ACTION DES AGENTS OXYDANTS. — Ils ont opéré en présence de NO_3H à 7 % et à 20 % brome et ozone.

a) L'action de l'NO_3H est consignée dans le tableau suivant :

$$C_{12}H_{18}O_9 \begin{array}{c} +7\% \ HNO_3 \\ \\ +20\% \ HNO_3 \end{array} \begin{array}{c} C_{24}H_{32}O_{11} \\ \text{acide} \\ \text{humique} \end{array} \longrightarrow \begin{array}{c} C_{23}H_{23}O_{12}NO_2 \\ \text{acide} \\ \text{insoluble} \\ (C_{23}H_{24}O_{12}) \\ \\ (C_{23}H_{24}O_{13}) \end{array} + \begin{array}{c} C_{11}H_{15}O_8NO_2 \\ \text{acide} \\ \text{soluble} \\ \\ \text{isolé par} \\ \text{dihydrazone} \end{array}$$
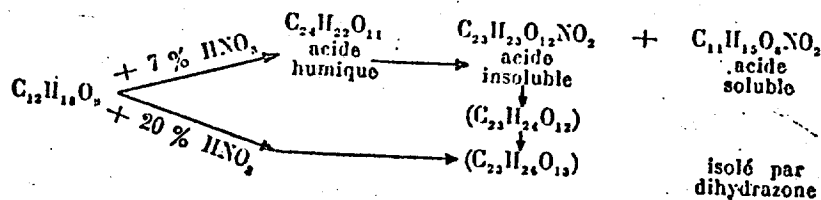
L'action du brome détermine l'obtention des deux produits ci-dessous :

$$C_{33}H_{53}O_{24} \ Br \quad \text{et} \quad C_{35}H_{52}O_{24}, \ Br_2,$$
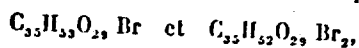
le premier exigeant 7.9 % de brome, le second 14,6 % de brome.

Les auteurs rapprochent ces composés du sesquibromo-oxysacchulmide obtenu par Sestini [33].

Avec l'ozone ils caractérisent l'existence d'un composé insaturé $CH_3CH : CX$. L'ozonide est décomposé par l'eau en $CH_3CHO + X \ COOH$.

Leurs conclusions sont que :

1° Le sucre chauffé à 170-180° perd deux molécules d'eau pour donner de le Caramélane $C_{12}H_{18}O_9$, tétra alcool p.f. 136°, caractérisé par un tétra acétate p.f. 107°, un tétrabenzoate p.f. 105°, un tétranitrate explosif.

Formule : $C_{12}H_{18}O_9$ ou $C_{24}H_{36}O_{18}$.

2° Les produits formés par la Caramélane avec la phénylhydrazine et la semi-carbazide indiquent l'existence du groupe CO et CHO par C_{24}.

3° Le traitement par les acides non oxydants donne des produits de déshydratation et hydrolise : dextrose, méthylfurfurol, aldéhyde, et acide humique.

4° L'oxydation donne des substances complexes.

5° La Caramélane est le premier pas dans le procédé de formation anhydre et de condensation du simple sucre à la cellulose, l'humus et la Caraméline.

Par ailleurs, contrairement à Stolle, les auteurs ont trouvé que le poids moléculaire de la Caramélane dans l'eau est le double de la composition moléculaire, que le tétra-acétate donne un poids moléculaire double dans C_6H_6, et le tétrabenzoate un poids quadruple.

En 1924, Pictet [34] a également traité le problème du caramel. Il a reproché à tous les auteurs qui l'avaient précédé de n'avoir pas effectué leurs recherches sur des produits purs. Il basait son affirmation sur le fait que les matières analysées étaient fortement colorées. Leurs analyses étaient inexactes et leurs résultats concernant les points de fusion sensiblement différents. Il procéda à une caramélisation « sous vide » afin que les produits acides prenant naissance en cours de fabrication soient rapidement séparés. En chauffant dans « le vide » à 180° jusqu'à une perte de poids de 5 % il obtint un produit qu'il appela isosaccharosane. Ce produit, dissous dans

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l'alcool méthylique, était précipité par addition d'acétone et donnait une poudre amorphe. Il lui donna pour formule $C_{12}H_{20}O_{10}$.

Très soluble dans l'eau où il se transforme en sucre interverti, ce produit fond aux environs de 94°. On obtient un hexa-acétate de p.f. 79/80° en présence de pyridine et d'acide acétique anhydre. Le pouvoir réducteur sur le Fehling est la moitié de celui du glucose. Pictet en conclut qu'en chauffant au-dessus du point de fusion du saccharose, ce dernier se transforme d'abord en isomère et que c'est par la suite que se produit la déshydratation. Le chauffage prolongé dans « le vide » conduisit Pictet à l'obtention de la Caramélane de formule $C_2H_{36}O_{18}$, p.f. 144/145°. En prolongeant le chauffage, il obtient la Caramélène de formule $C_{36}H_{50}O_{25}$, d'un p.f. de 104/106°.

Nous citerons également la thèse du docteur A. Schweitzer [35], qui constitue un document de synthèse bibliographique extrêmement complet. Reproduisant, parachevant, contrôlant l'ensemble des travaux effectués sur le Caramel, c'est à notre connaissance le dernier document respectant les critères traditionnels de l'analyse du caramel. Il pense que « le caramel est un mélange d'isosaccharosane de Pictet et d'humine, cette dernière étant un hydrate de carbone sous une forme polymérisée très poussée ». Il ajoute que « l'humine est insoluble dans l'eau et qu'en présence d'une certaine quantité d'isosaccharosane la réunion de ces deux produits devient soluble ».

Le doute et la négation animeront les recherches de Joszt et Molinski [36] et celles de Cruisheer [37].

Joszt et Molinski arrivent à la conclusion qu'il n'existe ni Caramélane, ni Caramélène, ni Caraméline. Ils ont recherché ces trois substances dans l'eau de dialyse. Ils ont étudié leur degré d'acidité, leur tension superficielle, leur viscosité, leur intensité colorante, qu'ils considèrent comme très voisines. Ils estiment qu'il existe de très petites différences dans leur constitution élémentaire.

Cruisheer considère que non seulement tous les produits précédemment niés par Joszt et Molinski sont fictifs, mais il nie également l'existence de l'isosaccharosane.

Von Elbe [38] considère le caramel comme un mélange de deux produits se rapprochant du saccharose, l'un des produits étant constitué par une dispersion colloïdale d'humine.

Schweitzer note « que l'acétate d'un des produits a une composition correspondant à la formule d'un hexa-acétate de l'isosaccharosane ».

De très nombreux auteurs ont mentionné au cours de publications la composition des produits de caramélisation.

Janeck [39] considère, par exemple, que la formule de Sabanejeff [40].

$$11\ C_{12}H_{22}O_{11} = 7\ CO_2 + 27\ H_2O + C_{125}H_{188}O_{80}$$
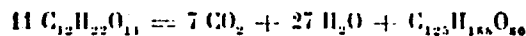
était meilleure que celle de Gélis pour la Caraméline obtenue par dialyse.

Brown et Zervan [41] donnèrent le nom de saccharane au produit précédemment appelé par Ehrlich saccharone. « Une partie de ce corps colore 10.000 parties d'eau en brun foncé qui peut être augmenté par addition d'alcali. Il a moins de goût et n'est pas précipité par le sous-acétate de plomb comme les matières colorantes du sirop. » A l'épreuve du temps, ces recherches apparaissent comme fantaisistes.

Les précisions acquises à cette époque permettaient aux savants de rattacher le caramel aux dérivés humiques issus des sucres, ce qui constituera l'objet de notre deuxième chapitre.

CHAPITRE II

Comportement des sucres sous l'influence de certains facteurs chimiques ou physiques.

La documentation dans ce domaine est extrêmement abondante. Nous insisterons plus particulièrement sur les travaux présentant un intérêt pour nos études et d'abord liés à des recherches sur les hydrates de carbone. Tous ne seront pas mentionnés, une telle besogne nous entraînerait dans une véritable bibliographie des recherches sur l'humus. Si l'on tient compte en effet que les matières humiques se forment par « décomposition des hydrates de carbone sous l'action d'acides étendus ou concentrés, par l'hydrolyse de matières albuminoïdes ou par l'action oxydante des alcalis sur les polyphénols », on conçoit que notre exposé soit limité.

Par ailleurs, nous respecterons dans une large mesure l'ordre chronologique des travaux. Il nous arrivera de transgresser cette règle pour satisfaire aux exigences créées par le courant des techniques ayant guidé les auteurs.

Nous croyons utile dès le début de cet exposé de familiariser le lecteur avec les termes que nous allons rencontrer, notamment ceux afférents aux matières humiques.

Tout le monde s'accorde pour considérer la communication de Sven Oden [42], dans *Kolloïd. Beih. II*, p. 75-200, comme un élément de base de cette classification.

En 1889, Hoppeseyler [43] ayant déterminé l'acide hymatomélanique, les matières humiques se classaient déjà en acide humique caractérisé par Berzelius [44] en acide fulvique caractérisé par Oden, ledit acide fulvique se décomposant en acide Creen et Apocreen de Berzelius. Cette classification devait être achevée en 1938 par Springer [45], qui distinguait les acides humiques bruns et gris, lesquels, étant ajoutés à l'acide hymatomélanique de Hoppeseyler [43], reconstituaient l'acide humique de Berzelius. Nous schématiserons cet ensemble dans le tableau ci-dessous :

Matières humiques.

Acide fulvique		Acide humique		
Acide Creen	Acide Apocreen	Acide hymatomélanique	Acide humique brun	Acide humique gris
faiblement jaune	jaune brun	brun	brun foncé	gris noir

Dès 1786, F. K. Achard [46], le créateur de l'industrie de la betterave, avait obtenu de l'acide humique en partant de la tourbe par extraction alcaline et précipitation acide (SO₄H₂).

Dobereiner [47] utilisa le premier l'expression « acide humique ».

C'est en 1819 que Braconnot [48] obtint le premier des produits humiques artificiels à partir du sucre sous l'action des acides.

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Thomson [49], ayant traité l'écorce d'*Ulmus Nigra*, obtint par l'extraction alcaline et précipitation acide un produit qu'il appela l'ulmine. Durant un certain temps, on utilisera les termes acide humique et ulmine.

En 1839, Berzelius [44] donna de l'humine la formule $C_{52}H_{52}O_{16}$. Oxydant la tourbe par NO_4H, il obtint deux dérivés acides à qui il donna les noms d'acide Creen et Apocreen.

Malaguti [50], en 1835 et 1836, amorça les travaux qui furent repris plus tard par d'autres auteurs.

En 1840, Mulder [51] obtint avec le sucre par l'action des acides dilués (SO_4H_2, HCl) quatre corps, dont deux solubles dans les alcalis et deux insolubles. Deux sont marron : l'un de ceux-ci, l'acide ulmique, est soluble, sa formule $C_{40}H_{24}O_{12}$; l'autre, insoluble, est appelé ulmine, formule $C_{40}H_{32}O_{14}$. Les deux autres sont marron très foncé ou noir. Il les appelle humine, formule $C_{40}H_{30}O_{15}$, et acide humique $C_{40}H_{24}O_{12}$.

En 1875, Tollens et Grotte [52], en 1886, Conrad et Guthzeit [53], poursuivirent des recherches sur l'action d'SO_4H_2 et d'HCl sur différents sucres et obtinrent de l'humine, de l'acide lévulique, de l'acide formique, de l'acide lévulique permettant de conclure que la formation de l'acide lévulique et celle des matières humiques étaient simultanées. Ils avaient traité les sucres durant 15 à 20 heures, les chauffant avec des acides étendus et séchant l'humine à 130°. Dès ce moment, ils avaient constaté que certains sucres étaient beaucoup plus sensibles que d'autres à l'action des acides.

De 1892 à 1905, Berthelot et André [54], dans une série de communications, confirmèrent et précisèrent ces résultats. Ils traitèrent le dextrose, le lévulose, la galactose et le maltose avec les acides HCl SO_4H_2, PO_4H_3, plus ou moins étendus.

Leurs expériences se firent :

a) en tubes scellés où l'on fait le vide, maintenus à 100°;

b) des ballons chauffés en bain d'huile :

1° en condensant l'eau avec un réfrigérant qui ramène les produits volatils au sein du liquide;

2° à l'aide d'un réfrigérant descendant avec renouvellement de l'eau perdue.

Les expériences furent faites sous vide, à l'air, sous atmosphère d'hydrogène.

On a dosé :

1° le glucose inaltéré (dosage imparfait au Fehling à cause de la présence de glucosanes);

2° CO_2 (eau de chaux);

3° CO (chlorure cuivreux acide);

4° acide humique (par lavages, dessiccation à 100° et pesée);

5° acide formique;

6° acide lévulique (méthode des coefficients de partage);

7° furfurol (par la phénylhydrazine);

8° l'eau.

Dans une deuxième communication, Berthelot constate que les acides humiques solubles dans les alcalis se transforment, par chauffage avec HCl à

10 %, en humine insoluble dans l'alcali, et par la suite il étudia l'action de NH_4OH sur l'acide humique, l'exprimant par la réaction :

$$3 C_{18}H_{18}O_7 + NH_3 = C_5H_{18}O_{15}N + 2 H_2O.$$
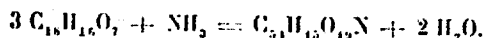
La présence d'azote avait déjà été signalée en 1871 par Delmer [55] dans l'humine naturelle (1 à 2 %).

Dès 1912, Maillard [56] devait apporter une explication extrêmement féconde dans ce domaine et exposer sa théorie au cours de différentes communications. Il étudia la réaction du glycocolle sur le glucose — 1 partie du glycocolle, 1 de glucose, 3 à 4 parties H_2O pour faciliter la solution. Il chauffe au bain-marie. Le liquide se teinte en jaune au bout de 10 minutes, puis sa coloration augmente pour devenir foncée. Il y a dégagement de CO_2. Celui-ci résulte « de la scission du carboxyle du glycocolle; si l'on admet que cette scission est corrélative de la fixation de N sur le carbone aldéhydique du sucre, les molécules du glucose, au nombre de 2 au moins, qui entrent dans la constitution du nouveau corps éprouvent des déshydratations qui créent des doubles liaisons et peut-être des cycles ».

Les substances formées pourraient être des molécules polycycliques à un atome de N.

Il a traité le glucose par le glycocolle, la sarcosine, l'alanine, la valine, la leucine, la tyrosine, l'acide glutanique.

L'alanine est le plus actif des aminoacides.

Le xylose et l'arabinose réagissent instantanément; fructose, galactose, glucose, mannose, assez rapidement; le lactose et le maltose, lentement.

Réaction violente à 150°, rapide à 100°, quelques jours à 37°. Cette réaction s'exprime par le schéma suivant :

$$CH_2OH (CHOH)_4 CH : O + H_2N CH_2 COOH = CH_2OH (CHOH)_4 CH : N CH_2 COOH + H_2O$$

$$CH_2OH (CHOH)_4 CH : N CH_3 + CO_2$$

$$CH_2OH (CHOH)_4 CH : O + H_2N CH_2 COOH = CH_2OH (CHOH)_3 NH CH_2 COOH$$

$$CH_2OH (CHOH)_3 NH CH_3 + CO_2$$
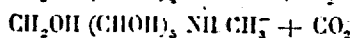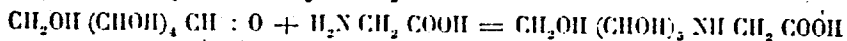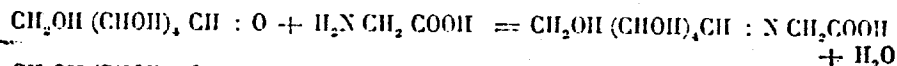
Il notait ultérieurement que les substances brunes formées dans ces expériences « ressemblent à celles qui prennent naissance dans la caramélisation des sucres ». La teneur en azote, 4.35 % à 6 %, était du même ordre que celle de certaines matières humiques extraites du sol et des produits mélanoïdiques, d'où il concluait à la relation étroite de ces substances.

Il précisait, en outre, « que l'oxydation n'intervenait en aucune façon dans la production de CO_2 et des matières humiques. Il ajoutait que « la genèse des combustibles minéraux pouvait aussi relever dans une certaine mesure de cette réaction ».

Etudiant enfin « la formation des matières humiques par l'action de polypeptides sur les sucres », il notait que « la chaîne des polypeptides, quelle que soit sa longueur, se termine toujours par deux extrémités libres NH_2 et COOH : il est intéressant de savoir si les groupements intermédiaires empêchent ou non cette molécule de réagir à la façon d'un simple acide aminé ».

Il prit d'abord un dipeptide, la glycyl-glycine :

$$H_2N — CH_2 CO NH CH_2 COOH$$
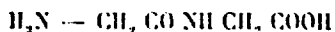
dont les échantillons contrôlés purs provenaient de l'hydrolyse partielle de la cyclo-glycyl-glycine obtenue par sa méthode, et les mit en présence de xylose, ce dernier étant choisi pour sa remarquable sensibilité.

Dans un petit vase maintenu à 75° on place 0,5 de glycyl-glycine, 2 grammes de xylose et 3-4 cm³ d'eau. Au bout de 10 minutes, la coloration jaune est déjà bien nette; elle est forte après 10 minutes, presque brune après 20 minutes, d'un brun noir après 23 minutes. Après 1 heure 50 minutes, on observe déjà de fines bulles de CO₂ dans la masse qui, au bout de 2 heures, est abondamment vacuolisée. Les phénomènes se poursuivent comme dans le cas du glycocolle; au bout de quelques heures, on voit s'insolubiliser une partie du produit qui se transforme tout entier en une masse de pellicules insolubles si on le maintient sur le bain-marie pendant 2 ou 3 jours en renouvelant la petite quantité d'eau lorsqu'il est nécessaire.

« La matière brune ainsi formée se comporte exactement comme celle qui dérive du glycocolle. »

« La réaction se produit aussi à température plus basse, bien qu'avec plus de lenteur : à 40° il faut environ 24 heures pour donner au mélange la coloration brun foncé; à 34° environ, 40 heures sont nécessaires. »

Il obtient les mêmes résultats avec le glucose et la glycyl-glycine, bien que plus lentement. Le mode opératoire est rigoureusement semblable. Il ajoutait que les polypeptides étaient capables d'intervenir de la même façon que les amino-acides. Maillard avait noté précédemment que la réaction de déshydratation s'effectuait à une température considérablement plus basse en présence des amino-acides qu'au cours de la caramélisation. Ce que l'on constate lorsque l'on effectue celle-ci en présence de sels ammoniacaux ou d'ammoniaque, et à ce sujet nous citerons tout de suite une communication de Ling et Nansit [57] quoiqu'elle date de 1922.

Les auteurs ont étudié l'action de NH_3 sur les sucres en C_6 (glucose et lévulose).

Quand on traite le glucose par NH_3 à faible température : 35, 40° environ et pendant assez longtemps, il se formerait une combinaison de glucose et de NH_3, appelée glucose ammoniaque. Si, ensuite, on porte cette combinaison à la température de 100° environ, il se produit une réaction exothermique et des substances noirâtres très colorantes apparaissent. Cette combinaison glucose-ammoniaque se produirait également par l'action des amino-acides sur le glucose, et elle permettrait d'expliquer la coloration des malts bruns obtenus par chauffage à faible température du malt, qui sont utilisés dans la fabrication de la bière brune.

La réaction glucose-ammoniaque serait la suivante :

$$
\begin{array}{lcl}
CH(OH)_2 & \cdots\cdots\cdots\cdots & CHOH - NH_2 \\
| & & | \\
CHOH & & CHOH \\
| & & | \\
CHOH & + NH_3 & CHOH + H_2O \\
| & & | \\
CHOH & & CHOH \\
| & & | \\
CHOH & & CHOH \\
| & & | \\
CH_2OH & & CH_2OH
\end{array}
$$
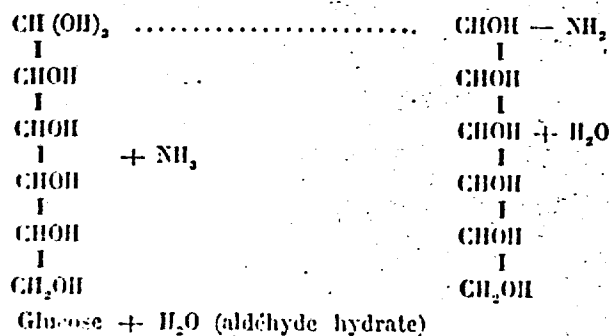
Glucose + H₂O (aldéhyde hydrate)

Cette réaction serait réversible; l'allure de la réaction doit dépendre de la concentration en glucose de la solution utilisée et de la concentration en NH_3; d'après l'auteur, il faudrait une solution à 20 %.

La même réaction se produirait avec le lévulose, mais à température plus basse et en chauffant à 62°-76° des substances brunes apparaissent.

En 1915, Bottomley [58] étudia la formation des humines dans le traitement du glucose et du lévulose à chaud. Chauffant du glucose pur durant 4 heures à 180°, il obtint des produits colorés en marron clair parfaitement solubles dans l'eau ou par addition d'acides forts il précipita des humines insolubles. En élevant la température à 220° et en chauffant durant 2 heures. le produit obtenu n'était plus soluble que dans une lessive alcaline diluée. Ledit produit précipitait cependant par addition d'HCl. Traitant le fructose, il constatait qu'après 6 heures de chauffage à 120° le produit obtenu de couleur marron foncé était soluble dans l'eau, mais précipité par HCl. Après 2 heures de chauffage à 120°, le produit n'était plus soluble que dans les alcalis. Si le chauffage était élevé à 200° durant 2 heures, l'humine obtenue était uniquement insoluble dans l'alcali. Il notait que le lévulose était plus sensible que le dextrose à l'élévation de température, ce que l'on connaissait déjà.

En 1920, Fisher, Schrader et Treiss [59] transformèrent par oxydation en milieu alcalin grâce à l'oxygène de l'air et à une température élevée l'humine de sucre, diverses sortes de houilles et la cellulose.

Pour ces deux derniers, il avait opéré à 250° de température et 50 AE de pression. Pour la houille, ils obtinrent de l'acide benzoïque, isophtalique, des acides carbobenzoïques, des benzols penta-carboxylés. Pour la cellulose du CO, du CO_2, de l'acide furanique et des traces d'acide oxalique.

Avec les sucres, la précédente technique leur avait donné un solution rouge brun, limpide, dans laquelle les acides ne précipitaient aucun corps. Ils élevèrent la température à 400° et obtinrent dès lors du CO, du CO_2, du furane, des acides aromatiques, benzoïques et des dérivés phtaliques. Ils conclurent que la condensation des groupes aldéhydiques et cétoniques en noyaux aromatiques s'effectuait de la même manière que l'on obtient le mésétylène en partant de l'acétone (Kane, 1838).

Eller [60], à la même époque, commença une série de recherches dans laquelle il utilisa NO_3H concentré. Son but était de montrer la différence entre les humines de phénols et les humines de sucres. Traitant à froid l'humine d'hydroquinone par NO_3H, il obtint une matière soluble dans l'alcool, l'acétone, l'éther éthylique, mais insoluble dans les autres solvants organiques. Ce corps était très hygroscopique. Avec l'eau on obtenait la réaction :

$$2 (C_6H_4O_3) X + (H NO_3) X = (C_{12}H_7O_6NO_2) X + (H_2O) X.$$
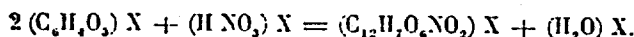
Avec NO_3H étendu à chaud il obtenait des produits mal définis. Vis-à-vis d'NO_3H, la stabilité de l'humine de sucre est plus considérable que celle de l'humine du phénol. L'humine de sucre se dissout à température normale dans NO_3H, avec dégagement de vapeurs rutilantes. Après filtration, la fraction non attaquée est plongée dans l'eau. Elle est soluble dans l'alcool et partiellement dans l'éther. C'est une poudre jaune amorphe, et sa dissolution immédiate donne :

$$(C_{21}H_{21}O_9) X + (2 HNO_3) \infty = (C_{21}H_{21}O_{14}N_2) X + (H_2O) X.$$
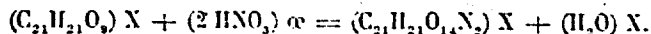
Si l'on élève la température, l'humine est décomposée. Avec l'humine d'hydroquinone on obtient de l'acide oxalique en assez forte quantité. Avec l'humine du sucre il y a des traces d'acide oxalique formées.

En 1921, Marcusson [61], qui s'était révélé déjà au monde scientifique comme un technicien des produits humiques par une communication en 1919, fit une découverte qui, quoique légèrement éloignée des thèses exposées par Midendorf [62] en 1917 au sujet du rôle de l'oxyméthylfurfurol, dans

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la composition des produits humiques, devait l'entraîner à soutenir l'idée d'une structure furanique de l'humine de toute origine.

Marcusson, ayant traité le furfurol par HCl concentré, obtint une masse noire pratiquement insoluble dans tout. En la fondant avec KOH à 250°, il obtint ClK et de l'acide humique. La démonstration était faite que le furfurol avec HCl se condensait en substances humiques. Williams Hoppeseyler et Koch [63] avaient constaté le dégagement du furfurol dans le traitement de la cellulose. Le furfurol est d'ailleurs obtenu en traitant le son par SO_4H_2 dilué.

Beckley [64], en Amérique, obtint à la même époque des résultats semblables, mais avec l'oxyméthylfurfurol.

En 1923, Burian [65] crut pouvoir démontrer la présence des dérivés du furane et du furfurol dans les distillats des acides humiques naturels ou artificiels obtenus respectivement par le brun de Cassel et la cellulose.

Marcusson pensa que les hydrates de carbones issus de la dégradation des résidus végétaux donnaient du furfurol. Ce composé donnait des groupements benzoïques par l'intermédiaire d'un noyau paradifurfuranique. On schématisa la réaction de la façon suivante :
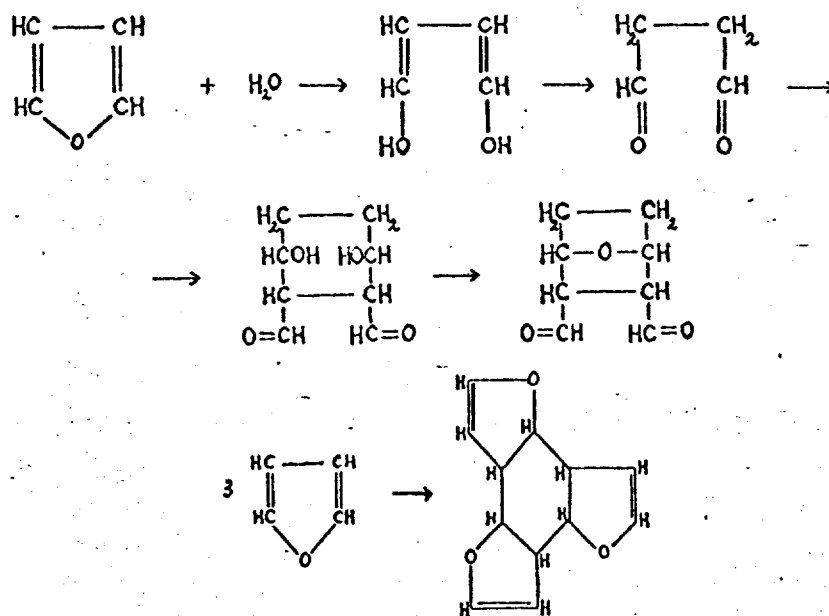
La matière noire obtenue par Marcusson [66] avait reçu le nom d'acide caramélique, et il est inutile de préciser que ses recherches ultérieures furent toujours influencées par le souci de démontrer la présence de ce corps. Il utilisa notamment l'eau oxygénée avec des traces de SO_4Fe pour l'obtenir en partant de l'acide humique de Merck.

Francis et Wheeler [67] étudièrent l'action de l'eau oxygénée sur la houille en solution alcaline.

Ils obtinrent des produits solubles dans l'alcool, l'acétone et la pyridine. Bone, Horton et Ward [68], à la même époque, oxydèrent la houille par le permanganate de potasse, obtenant de l'acide humique, de l'acide oxalique, du CO_2 et du benzol polyacide.

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En 1926, Fuchs [69] et ses collaborateurs commencèrent une série de travaux d'une importance extrême. Ils utilisèrent les produits nitreux pour mettre en lumière la constitution des produits humiques. Ils étaient d'avis que les liaisons azotées se trouvaient en relation étroite avec l'humine elle-même.

Ils donnèrent le nom d'acide déshydrohumique au corps obtenu dans leurs recherches et rangèrent les acides hymatomélaniques naturels dans cette catégorie. Ils établirent le poids moléculaire de cette substance grâce à sa solubilité dans l'acétone.

En procédant à la nitratation ils constatèrent une augmentation de 2 à 3 % de la teneur en N. Ce chiffre se rapproche de la teneur en N de l'acide humique naturel, qui est de l'ordre de 3 à 5 %.

Fuchs pensa qu'il se formait alors des groupes isonitrosyliques. Après plusieurs ébullitions en présence de HNO_3, Fuchs et Stengel [70] obtinrent, avec un peu d'acide oxalique, plusieurs acides à noyaux benzéniques, avec 4 à 5 groupes carboxylés, acide picrique et d'autres nitrophénols. Fuchs essaya de méthyler complètement le produit nitreux en le chauffant d'abord avec HCl et l'alcool méthylique et ensuite en faisant agir un diazométhane. Il a recherché les groupes présents et obtint la formule :

$$C_{39}H_{36}O_{17}N (COOCH_3) 4 (OCH_3) 3 (C = NOH) (CO).$$
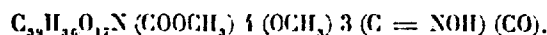
Il conclut à la présence dans les acides humiques naturels de :

    4 groupes COOH;
    4 groupes OH;
    1 groupe CH_2CO;
    2 groupes O;
    1 groupe CH : CH;
    1 groupe H substituable;
    4 à 8 groupes H déshydratables.

La préparation des éthers oxydes méthylés des fonctions alcooliques des humines de sucre a été faite par Fuchs [71], et il a comparé les divers acides humique en ce qui concernait les NH_3 absorbés et les relations à partir des NH_4 transformables en NH_3.

Ces comparaisons furent faites entre l'acide humique de Merck, l'acide humique de sucre et l'acide humique d'hydroquinone.

Les humines naturelles et les humines de sucre sont plus voisines en ce qui concerne leurs propriétés que les humines naturelles et les humines de polyphénols.

Outre l'action oxydante, l'action des halogènes, celle des groupements halogénés, fut utilisée pour mettre en lumière la similitude des humines naturelles, de sucre et de phénol. Fuchs [72] et Eller [73] ont étudié l'action de Cl et Br. Schmidt et Atterer [74] étudièrent l'action de ClO_2 avec un sel de vanadium comme catalyseur, sur les produits humiques en solution alcaline. Ils obtinrent de l'acide maléique attribué à un groupe — C — CH : CH C ou — C : CH — CH : C —.

Ces C_4 combinés se trouvent dans les noyaux C_6H_6 ou furanique.

Le furfurol traité par HCl concentré à froid donnait presque aussitôt un dégagement d'humine que l'on pouvait déceler avec ClO_2.

En 1927, Schmidt et Atterer [74] pensaient qu'il existait une énorme différence de structure entre les humines de furane et celle des autres catégories, et ils se sont demandé si les humines de sucre pouvaient être considérées comme dérivés du furane, étant donné que la transformation du furfurol en

humine pouvait être soit le résultat d'une simple polymérisation, soit le résultat d'un changement notable.

La question devait être reprise plus tard par Page et Du Toit [75] en 1932.

Berl et Schmidt [76] ont recherché la formation de charbon à partir de cellulose et glucose en chauffant avec de l'eau et sous pression à 225° et à 250°. A 250°, ils ont obtenu 27 % d'humine et 73 % de charbon.

Willstatter et Kalb [77], en milieu réducteur à 250° ont obtenu avec l'humine de glucose, la cellulose, le glucose et le xylose des résultats identiques.

Orlow et Tichenko [78] ont étudié les charbons de sucre en procédant à 170 AE de pression et à la température de 300°, puis à 100 AE et à 400/410° de température. Puis ils ont procédé à l'action de NO_3H sur ce produit.

Parallèlement à ces recherches, l'établissement de plus en plus précis des caractéristiques physiques des produits obtenus, et notamment des poids élémentaires par pression osmotique (Samec [79], C. L. Arnold [80]) diffusion (Zeile [81], Enders [82], Schelle [83]), cryoscopie dans l'acétone (Fuchs [84], Eller [85], Stach [86], Kurschner [87], basicité, constituait une armature des plus solides de l'étude des substances humiques, voire des tests analytiques.

Cet exposé nous a conduit à une date extrêmement voisine de celle qui clôt le chapitre I. La raison en est due au fait que, depuis 1900, les recherches sur le caramel faisaient une part toujours plus grande aux matières humiques. Les procédés de détection utilisés s'inspiraient directement des communications des techniciens de la chimie du sol ou du charbon (action des acides dilués ou concentrés, des oxydants, des halogènes, des alcalis, etc.).

Lorsqu'on est parvenu, grâce à la concordance des résultats et à l'établissement de méthodes classiques, à démontrer et contrôler le mécanisme de la formation des matières humiques à partir des hydrates de carbone, il était compréhensible que l'étude isolée du caramel et des produits humiques issus du sucre était pratiquement terminée et qu'une explication scientifique valable ne pourrait être trouvée que dans le cadre des hypothèses et des recherches afférentes à la chimie de l'humus. C'est ce qui nous conduit à la troisième partie de notre exposé.

CHAPITRE III

Théories récentes s'attachant plus spécifiquement aux deux catégories commerciales actuelles des caramels.

Nous avons achevé nos deux précédents résumés aux environs de 1936. A cette époque, Selman. A. Wacksmann [88], fixaient à trois les théories, « essayant d'expliquer la formation des substances noires dans l'humus ».

L'humus était dû :

1° aux réactions des acides sur les hydrates de carbone.

Conception chimique se fondant sur les théories de Marcusson, Beckley, Burian.

Les sucres en C₆ donnant du furfurol, les sucres en C₆ de l'oxyméthylfurfurol. Cette théorie était combattue par Eller et Schmidt;

2° à la condensation des hydrates de carbone par les acides aminés ou polypeptides. Théorie de Maillard;

3° à l'oxydation des composés à noyaux benzoïques. Théorie de Hoppe-Seyler, Reinitzer [89] et de Eller.

Depuis cette époque, des travaux considérables ont été effectués, et nous emprunterons strictement notre documentation au remarquable livre de Fritz Scheffer et Bernhard Ulrich [90], qui expose le résumé des dernières théories à ce sujet. Elles se développent dans deux chapitres que nous reproduirons dans une large mesure.

Les auteurs rappellent que la préparation des matières humiques à partir des sucres ou de la cellulose a été réalisée soit par l'action des acides minéraux forts, concentrés dans des conditions déterminées, soit par chauffage (caramélisation), Stadnikow [91], Bergstrom [92], Thiele [93].

Les sucres et les acides uroniques (acides aldéhydides de formule générale : $HO_2C - (CHOH)_4 CHO$, dérivés des aldoses en C_6) formés en cours de réaction, donnent entre autres :

    1° des réductones;
    2° des groupements ɀ-oxycarboxylés;
    3° des dérivés furaniques.

Les réductones et les groupements ɀ-oxycarboxylés se forment surtout dans des solutions alcalines neutres ou faiblement acides. Les réductones ont des caractères acides (Enders [94]).

LES RÉDUCTONES. — Elles possèdent le groupement atomique ci-dessous :

LXIX (n = 0 1)
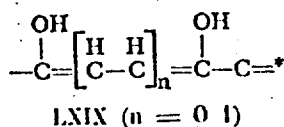
D'après Euler [95], « le premier terme de l'oxydation du groupement Endiol (LXX) serait l'abandon de 2 électrons, donnant ainsi naissance au biradical LXXI qui, par déplacement des électrons, se transforme en groupement dicétonique LXXII déshydroréductones, les dicétones sont fortement colorées en jaune. Cette formule fait apparaître une ressemblance avec le principe des réactions des phénoquinones ».

Les réductones ont une grande importance biologique. Le groupement « Endiol » existe dans les substances favorisant la croissance et ses inhibiteurs. Vitamines (acide ascorbique), hormones (adrénaline).
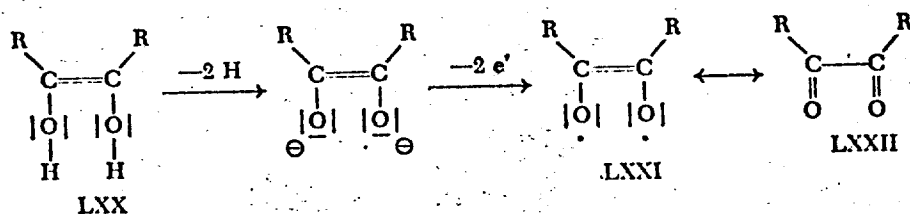
LES GROUPEMENTS ɀ-OXYCARBOXYLES véritables ou potentiels représentant les produits de dédoublement des sucres sont :

    $CH_2OH - CHO$ (aldéhyde glucolique);
    $CH_2OH - CHOH - CHO$ (aldéhyde glycérique);
    $CH_2OH - CO - CH_2OH$ (dioxyacétone);
    $CH_3 - CO - CO - CH_3$ (diacétyldicétone).

Ces produits sont susceptibles de former des matières humiques dans des solutions aqueuses.

Enders [96] a prouvé que le triose réducteur CHOH = COH - CHO était capable de synthèse cyclique, démontrant ainsi le développement des cycles quinoniques. Il a, en outre, donné un rôle primordial à un triose oxydo-réducteur vis-à-vis de l'aldéhyde glycérique, de la dioxyacétone et du méthyl glyoxal.

Flaig [97] propose le schéma ci-dessous :

En partant du méthylglyoxal ou propanalone (Aldéhyde cétone), CH₃ — CO — CHO (aldéhyde pyruvique) :

LXXVII
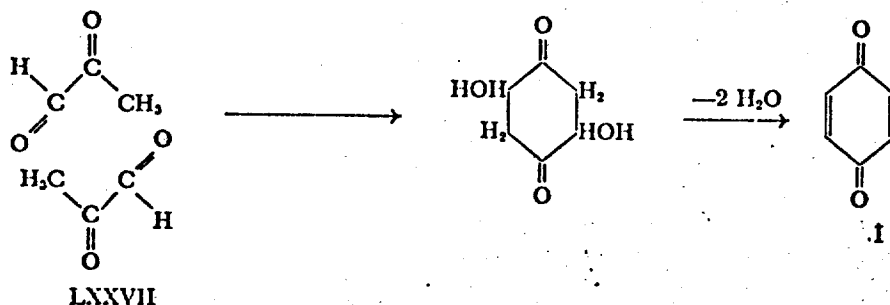
LES DÉRIVÉS FURANIQUES (Fisher [98], Marcusson [99], Eller [100]). — La polymérisation des sucres se produit déjà dans les solutions fortement diluées et peu acidifiées. Le furfurol forme avec l'oxygène de l'air un peroxyde. Tous les dérivés furaniques sont auto-oxydables sous l'influence de l'oxygène de l'atmosphère et donnent des produits bruns responsables de la coloration des mélasses.

Ces trois paragraphes épuisent, semble-t-il, nos connaissances actuelles dans le domaine de la caramélisation proprement dite ou pyrogénisation des sucres.

L'apparition au début du siècle de produits colorants issus du sucre, dans des conditions pratiquement définies par Salomon (A. G.) et Coldie (E. L.) [101], nous entraîne à exposer les théories récentes concernant la réaction de Maillard, à laquelle se rattache les corps obtenus dans ces conditions.

Scheffer et Ulrich [102] rappellent que ces produits précipitables en milieu acide sont des mélanoïdines. Les groupements aminés catalysent le dédoublement des sucres (selon que l'on considère les produits formés comme dus à une aldolysation ou à une désaldolysation) sous forme d'aldo-condensation ou de désaldo-condensation. On peut aussi fixer de l'azote dans ces produits réactionnels. Le rôle des groupements aminés se réduit à un effet catalytique. Ces conditions sont à peu près semblables à celles des groupements aminés réagissant sur les phénols.

Le dédoublement du sucre catalysé par des groupements aminés commence avec l'addition de RNH₂ à l'aldose existant (LXXXVI). Le corps d'addition LXXXVII se change en une base de Schiff en perdant de l'H₂O (LXXXVIII). Celle-ci se transforme en glycosylamine avec un N substitué IXC.

En passant par le cation de la base de Schiff (cation XC), il se forme : 1-amino-1-désoxy-2-cétose avec N substitué dans le groupe ENOL XIC, éventuellement dans le groupe Céto XIIC (se rapportant à la transformation [Amadori], qui est, comme on le sait, « la conversion des N glucosides d'aldose en dérivés aminés des cétones correspondantes » [103].

Dans la configuration 1, 2 ENOL, la liaison C — C en α ou β posi-

tion est affaiblie dans le groupe carboxyle. C'est pour cela que des groupements aminés catalysent la réduction d'aldoses en trioses et d'autres produits de dédoublement.

En partant de la 1-amino-1-désoxy-2-cétose XIIC, on arrive dans un milieu neutre ou acide aqueux par dédoublement de l'H_2O (catalysé par les acides) à la formation des bases de Schiff issues du furfurol LXXX ou de l'oxyméthylfurfurol LXXXII, desquelles on peut séparer en fixant de l'eau les groupements aminés.

Dans des systèmes sans eau, il se produit, par contre, de préférence des réductones par dédoublement d'eau.

Nous retrouvons dans les deux cas les produits de dédoublement des sucres dont nous avons déjà parlé (aldéhyde glucolique, glycérique, etc.).
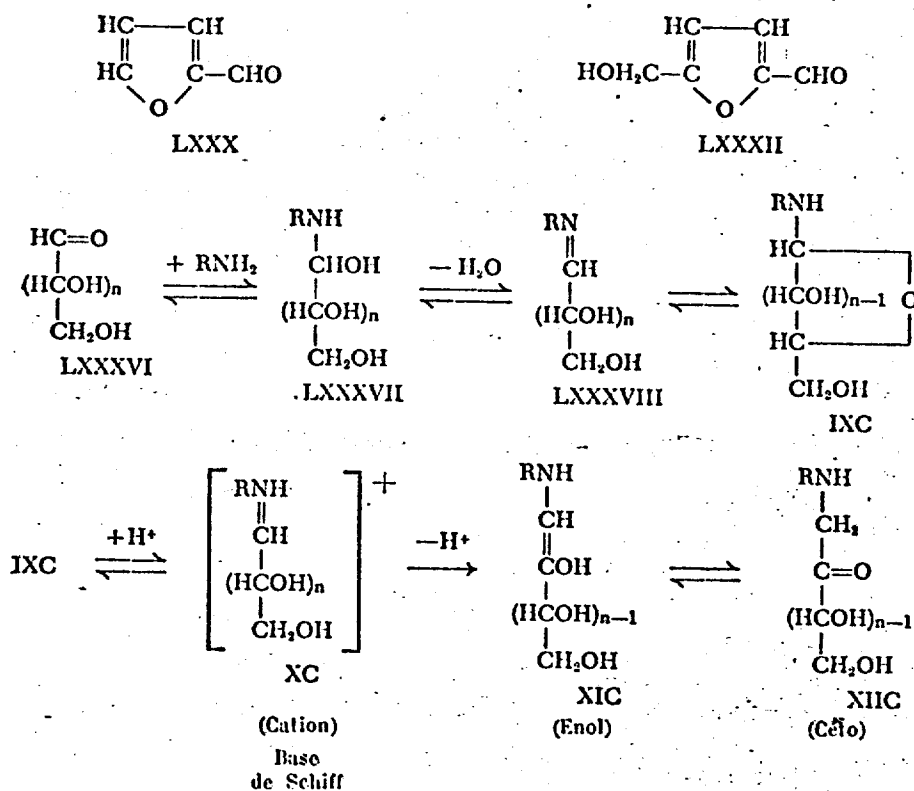
Concomitamment et dans une proportion importante, les amino-acides se transforment en aldéhydes correspondantes par réaction sur les groupements à liaisons dicétoniques avec dégagement de CO_2 (dégradation de Strecker) [104].

D'où le schéma :
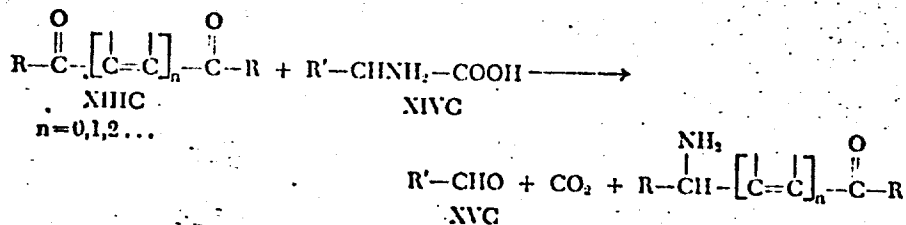
Dans tous les cas demeure une fraction osidique plus ou moins importante. Dans le cas où l'action thermique sur les sucres est conduite avec addition ménagée d'alcalis concentrés tels que KOH, NaOH, etc., la réaction terminale est pratiquement issue de la formation du triose réductone [105] :
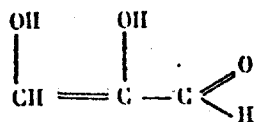
qui, en milieu aqueux, donne un système équilibré avec son tautomère de poids équivalent, l'hydroxymalonyldialdéhyde :
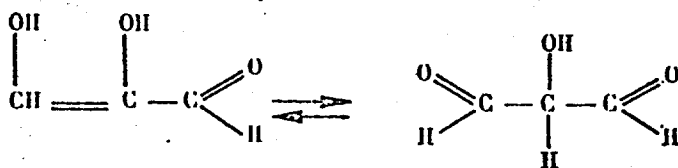
d'où prédominance des cycles quinoniques.

Dans le cas où l'action thermique a lieu en présence d'acides minéraux dilués (HCl, SO₄H₂, etc.) ou organiques (acide citrique, tartrique, etc.), nous avons formation [106] :

à partir des pentoses de furfurol·

à partir des hexoses d'hydroxyméthylfurfurol :
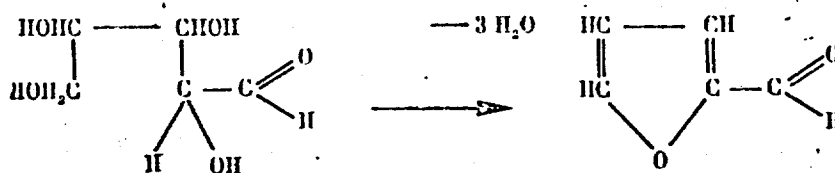
Pentose   Furfurol
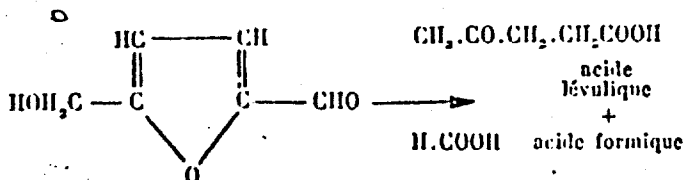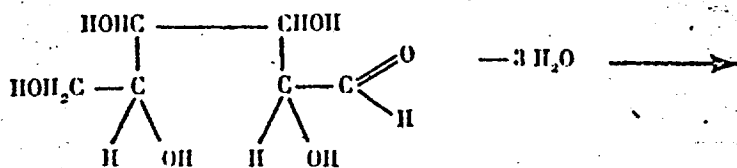
L'action thermique conduite de façon ménagée et en autoclave, en présence de NH₃ ou de sels ammoniacaux, donne lieu à la formation de corps issus de la réaction de Maillard, schématisée par Hodge [107] dans le tableau ci-dessous :
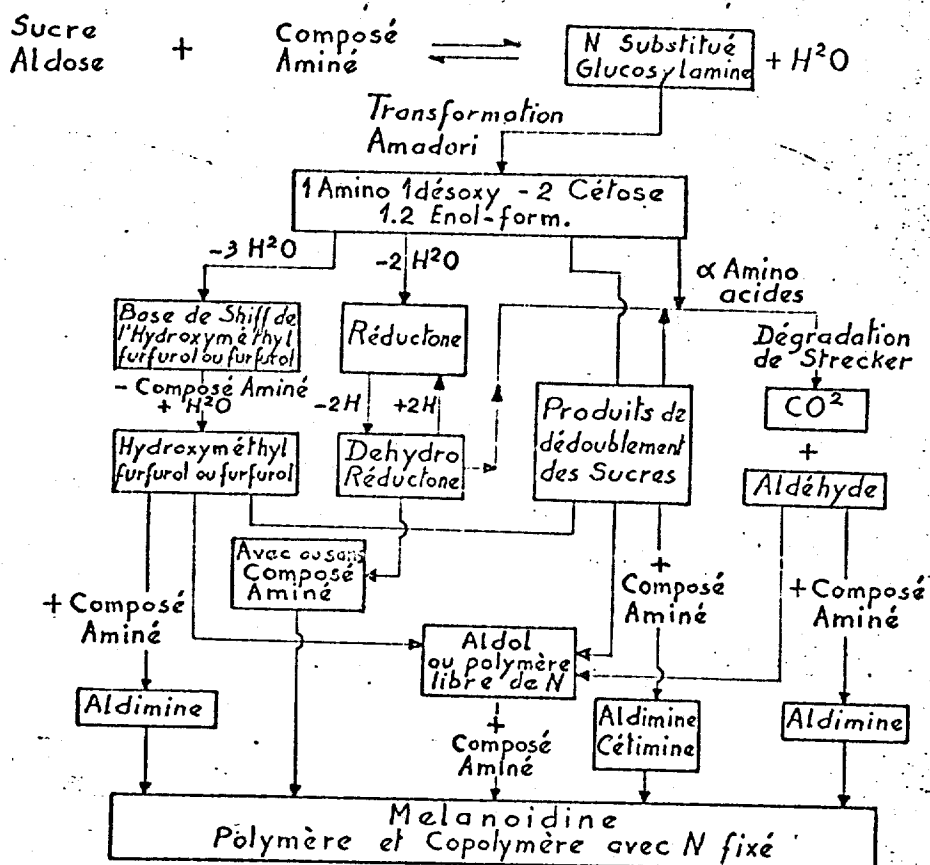

Le brunissement des matières alimentaires étant dû à quatre réactions principales [108] :

1° Oxydation enzymatique ou non des polyphénols;

2° Transformation thermique des protéines et de leurs éléments de base;

3° Pyrogénisation des hydrates de carbone;

4° Réaction de Maillard,

on constate que l'origine de la formation des produits composant les caramels est à rattacher à ces deux dernières réactions.

Les éléments exposés ci-dessus apportent donc des bases précieuses pour l'interprétation des phénomènes réactionnels assurant l'obtention des substances colorantes à partir des sucres.

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La formation de noyaux iso et hétérocycliques s'effectue en partant des réductones en présence des amines et aldéhydes selon le schéma ci-dessous :

$$R-C=O \quad + \quad NH_2R' \quad \longrightarrow \quad \begin{matrix} R-C--N \\ R-C \quad C-R'' \\ N \\ H \end{matrix}$$
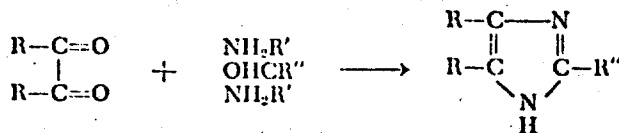
Conduisant à des noyaux pentagonaux et hexagonaux.

En résumé, la polymérisation des sucres par action thermique seule entraîne la formation :

1° de réductones;
2° de groupe α-oxycarboxylés;
3° de dérivés furaniques : quinone, furfurol, oxyméthylfurfurol.

Celle effectuée en présence de catalyseurs, tels que les acides aminés, donne :

1° des réductones;
2° des aldéhydes (issus de la dégradation des α-amino-acides);
3° des corps cycliques penta et hexagonaux : quinone, furfurol oxyméthyl-furfurol et des dérivés imino et amino-Pyrrol, Imidazole, Pyridine, Pyrazine.

Les résultats mentionnés sont cependant issus de recherches scientifiques éloignées des procédés industriels utilisés tant pour les quantités ou la qualité des produits mis en œuvre que pour les conditions opératoires.

Les techniques industrielles ont, elles aussi, fait l'objet de travaux et publications que nous nous proposons de développer dans une deuxième partie, qui comprendra également les principales réactions de détection et caractérisation, tant physiques que chimiques, des caramels.

Le rappel des brevets déposés à ce jour intéressant le matériel ou la fabrication des colorants issus des sucres complétera cette deuxième étude.

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REPORT ON CARAMEL IN ALCOHOLIC BEVERAGES

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The following recommendations were adopted last year by Committee
D, *This Journal* 32, 59 (1949).

That the Milos test for caramel, 14.35 and 15.38, be deleted (final
action).

That the Mathers test for caramel, *This Journal*, 31, 76 (1948), be
studied collaboratively with respect to its application to beer.

That the Mathers test for caramel. *This Journal* 31, 76 (1948), be
adopted as official (final action), for distilled liquors and wine and that

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* For report of Subcommittee D and action of the Association, see *This Journal*, 33, 59 (1950).

it be studied collaboratively, with respect to its application to cordials and liqueurs before adoption as final action.

During 1948, the Mathers test had been collaboratively studied and was accepted as an official test for caramel in wines and distilled liquors and it was recommended to take the place of the Milos test for caramel, which was to be deleted. The confirmatory portion of the Milos test however (15.39, p. 189), was not to be removed, but is intended to become the confirmatory part of the Mathers test. The purpose is to strengthen the test. A few minor changes and removals were made in the confirmatory test in which a number of small tests were deleted and the 2,4-dinitrophenylhydrazine reagent was used to confirm caramel in the final brown residue or its aqueous solution obtained by the confirmatory test.

Although the Mathers test for caramel was recommended in 1948 by the Associate Referee to be applied to beer, vinegar, cordials, and flavoring extracts, this could not be done under the requirements of the A.O.A.C. rules unless the recommendations for its acceptance as tests for caramel in these four classes of food products be supported by successful collaborative evidence and experiments. To this end the Associate Referee during the early months of 1949 following the Fall meeting of 1948 of the A.O.A.C., prepared substantial quantities of nine separate samples, and 8-ounce portions of each were submitted to a number of chemist collaborators, particularly those who by virtue of their analytical duties would be experienced in these classes of food substances. For the 20 actual sets of samples that were sent out, more than 25 separate chemists were involved and more than 200 actual tests were made.

Of the nine separate samples sent to each collaborator, samples 1, 2, 3, 7, 8, and 9 were analyzed correctly. About 80% reported correctly on samples 4 and 5 (beers) and about 90% reported correctly on No. 6, cordials; samples 1 and 2 were vinegar, sample 7 was beer, and 8 and 9 were vanilla extracts.

From the good results and the generally favorable comments of the collaborators, it is recommended that the Mathers test for caramel be included in the official methods of analysis of beer, vinegar, cordials and liqueurs, and flavoring extracts, but not to appear in detail as recommended in the analysis of wine, but by reference to the wine methods only. The method enclosed with the samples was as follows:

"*Mathers Test:* To 10 ml of the above samples previously introduced into a Babcock cream bottle, or any convenient small centrifuge bottle, add 1 ml of pectin solution (made by dissolving 1 gram of pectin in 75 ml of water and adding 25 ml of alcohol for preserving it). Shake well before using. Add to the material to be tested in the centrifuge bottle about 3 drops of concentrated HCl and fill bottle with alcohol (about 50 ml or more). Shake well and centrifuge for 5 to 10 minutes or more; and decant carefully the supernatant liquid off of the gelatinous residue. Dissolve the residue in the bottle by adding 10 ml water and shaking well. To this residue dissolved in water add about 3 drops of concentrated HCl and 50 ml or more of alcohol; shake

well and again centrifuge. Repeat this process until the upper alcoholic layer is quite clear and colorless. After the final decantation of the water-white supernatant alcohol, the gelatinous residue is dissolved in 10 ml of hot water. A colorless solution would show that no caramel is present. A clear brown solution is usually indicative of caramel coloring. To further confirm caramel, add 1 ml of the following reagent (made by dissolving 1 gram of 2,4-di-nitro-phenyl-hydrazine in 7.5 ml of concentrated sulphuric acid and bringing the volume up to 75 ml with 95% ethyl alcohol. Keep in a glass-stoppered bottle in which it will stay clear and stable for several months) to the residue dissolved in the 10 ml of hot water. Put the bottle in the beaker of boiling water for 30 minutes. In the presence of substantial quantities of caramel a precipitate forms almost at once. Smaller amounts show as a precipitate before the 30 minutes are up. Even the smallest amount of caramel will show a precipitate under the conditions described but if the analysis has been conducted as described above, no precipitate will appear if caramel is absent. In order to be sure a precipitate has formed, pour the hot test solution from the bottle on a small filter paper and wash any residue with hot water. A reddish brown precipitate will be clearly seen on the filter. This precipitate although amorphous is quite characteristic and will always be the same if caramel is present. A low-power microscope affords a fine examination of the precipitate. If caramel is found to be present by the above test, check the sample by the confirmatory test which involves the use of KOH and ZNCl₂ etc. If no precipitate is obtained by 2,4-di-nitro-phenyl-hydrazine reagent, report caramel as absent."

The following letter was written to each collaborator on or after March 29, 1919:

"There will be transmitted to you in the near future 9 samples consisting of beer, cordial, vanilla flavoring, and vinegar, which may or may not contain caramel coloring matter. You are requested to have these samples examined in your laboratory using the Mathers' method for the detection of caramel.

At the A.O.A.C. meeting in Washington last Fall, it was recommended that this method be accepted as an official method for the detection of caramel coloring matter in wine and spiritous liquors. It was suggested at that time that it might also prove satisfactory for the detection of caramel in beer, cordials, flavoring extracts, and vinegars; but before it is adopted as an official method, collaborative work should be performed for the purpose of ascertaining whether it will be satisfactory for detecting caramel in this class of product.

After you have tested the samples you will receive with the Mathers' method, compare the results obtained with other methods which may be available or are now being used for the detection of caramel in the products mentioned above. It would simplify matters if the Mathers' method could be used for detecting caramel in all of the products mentioned.

Kindly forward your report to the Washington laboratory not later than the first of September, with your comments and recommendations in such form that it may be presented by Mr. Valaer, the Associate Referee, at the next Fall meeting. A similar set of samples is being transmitted to each branch laboratory.

The Mathers' method is to be incorporated in the 7th Edition of the A.O.A.C. methods of analysis, which will be published in 1950 and will be used for the detection of caramel in all the products mentioned, if the collaborative work proves it to be satisfactory."